Biomarkers in Autosomal Dominant Polycystic Kidney Disease

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Zusammenfassung

Die autosomal dominante polyzystische Nierenerkrankung (ADPKD) ist eine der häufigsten monogen vererbten Erkrankungen, von der ~1 von 1000 Geburten betroffen sind, und somit ~750 000 Menschen in Europa. Das herausstechende Merkmal ist die Entwicklung und das kontinuierliche Wachstum von Zysten in beiden Nieren. Im Laufe des Lebens ersetzen die Zysten zunehmend das funktionelle Nierenparenchym und führen in 50% aller Fälle zwischen dem 50. und 60. Lebensjahr zum Erreichen eines terminalen Nierenversagens mit der Notwendigkeit die Nierenfunktion zu ersetzen. Die Forschung in der letzten Dekade erzielte neue Erkenntnisse zur ADPKD Pathogenese und verschiedene Medikamente wurden in präklinischen und klinischen Studien getestet. Trotz dieser Fortschritte, ist bis heute noch keine Therapie für ADPKD verfügbar.

ADPKD zeichnet sich durch eine grosse Heterogenität des Krankheitsverlaufes innerhalb und zwischen Familien aus. Die Gründe dafür sind bisher unbekannt. Traditionelle Parameter zur Evaluation des Krankheitsstadiums, wie die geschätzte glomerulare Filtrationsrate (eGFR), sind nicht geeignet um das Stadium der Erkrankung, vor allem zu einem frühen Zeitpunkt, exakt zu bestimmen. Ausreichend statistisch gepowerte Studien sind notwendig um neue Erkenntnisse über den Verlauf der Krankheit zu erhalten und um die Patienten zu diagnostizieren, die von neuen oder spezifischen Therapiemöglichkeiten am stärksten profitieren werden.

Im ersten Teil dieser Arbeit wird die Umsetzung einer internationalen Beobachtungsstudie von ADPKD Patienten in Europa beschrieben. Die EuroCYST Initiative dient als Plattform um die Pathogenese, Progressionsfaktoren, Mortalität, Komorbidität als auch gesundheitsökonomische Faktoren der ADPKD zu erforschen. Die Etablierung dieser Kohorte wird einen wesentlichen Beitrag zur Harmonisierung und Standardisierung des

Forschungsaufwandes in Europa beitragen und die Anwendung von neuen Technologien in der Behandlung von ADPKD sicherstellen.

Im zweiten und dritten Teil untersuchen wir potentielle Biomarker im Urin von ADPKD Patienten und testen diese auf ihre Eigenschaften das Krankheitsstadium und die Verlaufsprognose zu bestimmen. Unsere Ergebnisse weisen darauf hin, dass die Osmolalität, UACR und KIM-1 die Eigenschaft besitzen das Stadium der Erkrankung zu bestimmen. Hingegen NGAL, UMOD und CC16 qualifizieren sich nicht als Biomarker. Entsprechend unseres Wissenstandes, haben wir zum ersten Mal UMOD und CC16 in Urin von ADPKD berichtet. Makrohämaturie ist ein häufig berichtetes Symptom während des Verlaufs von ADPKD. Im Gegensatz dazu wurde mikroskopische Hämaturie nur selten untersucht. Mikroämaturie ist assoziiert mit verschiedenen Krankheitsindizes und könnte das Potential als weiterer Marker für das Erkrankungsstadium besitzen.

Zusammenfassend können wir mit unseren Studien neue Faktoren aufzeigen, die das Stadium der Erkrankung in der klinischen Praxis, möglicherweise genauer bestimmen als traditionell angewandte Parameter mit besonderem Fokus auf ein frühes Krankheitsstadium, in dem die Nierenfunktion noch nicht eingeschränkt ist und neue Therapieansätze mehr Nutzen für den Patienten haben werden.

Summary

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monogenic genetic diseases, affecting ~1 in 1,000 live births, around ~750,000 individuals in Europe. The most prominent feature is the development and continuous growth of bilateral renal cysts. During patients lifespan, cysts replace more and more functional renal parenchyma, ultimately leading to end-stage renal disease (ESRD) in 50% of the patients between their 50s and 60s. Research over the last decade brought new insights into the pathogenesis of ADPKD and various medical treatments were investigated in preclinical and clinical studies. Despite these advances, no disease modifying treatment for ADPKD is so far available.

ADPKD is characterized by a strong intra- and interfamilial variability in disease course, by yet unknown causes. Furthermore, traditional markers to assess disease state, like estimated glomerular filtration rate, are not appropriate to determine patients' disease state, especially in the early course of ADPKD. Adequately powered studies are needed to gain new insights in the disease course and to identify those patients that would benefit most from new or specific medical treatments.

In the first part of this thesis, we describe the implementation of a large international observational cohort of ADPKD patients in Europe. The EuroCYST Initiative will serve as a scaffold and platform enabling researchers to study the pathogenesis, progression factors, mortality, co-morbidity as well as health economic issues relevant to ADPKD as a major cause of kidney disease. The establishment of this cohort will help to harmonize and standardize research efforts in Europe and will guarantee that new technologies can be applied to ADPKD patients.

In the second and third part, we investigated potential new urinary biomarkers in ADPKD and tested their properties for the disease state assessment and prediction. Our results indicate that osmolality, UACR and KIM-1 may have the property to assess disease state during early

ADPKD disease course, whereas NGAL, UMOD and CC16 seem not to qualify as biomarkers. To our knowledge, urinary UMOD and CC16 levels in ADPKD have been reported by us for the first time. Macrohematuria is a commonly reported symptom during the course of ADPKD. In contrast, microscopic hematuria has been rarely investigated. Microhematuria is associated with various disease indices and may serve as potential marker for disease state.

In summary, these studies introduce and will promote possible new determinants to more accurately assess disease state compared with traditional markers in clinical routine, with focus on early disease, when kidney function is still within normal range and when potential new therapies would offer most benefit to patients.

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Abbreviations

ADPKD	autosomal dominant polycystic kidney disease
AKI	acute kidney injury
AVP	arginine vasopressin
cAMP	cyclic adenosine monophosphate
CC16	clara cell protein 16
CFTR	cystic fibrosis transmembrane conductance regulator
CKD	chronic kidney disease
eGFR	estimated glomerular filtration rate
ESRD	end stage renal disease
FJHN	familial juvenile hyperucemic nephropathy
GCP	good clinical practice
GFR	glomerular filtration rate
GPI	glycosylphosphatidylinositol
hrQoL	health related quality of life
htTKV	height adjusted total kidney volume
KIM-1	kidney injury molecule 1
LMWP	low molecular weight protein
LVH	left ventricular hypertrophy
MRI	magnetic resonance imaging
mTOR	mammalian target of rapamycin
NGAL	neutrophil gelatinase associated lipocalin
NKCC2	sodium-potassium-cloride transporter
PC1	polycystin 1

PC2	polycystin 2
PKD	polycystic kidney disease
PKD1	polycystic kidney disease 1
PKD2	polycystic kidney disease 2
PLD	polycystic liver disease
QoL	quality of life
RAAS	renin-angiotensin-aldosteron-system
ROMK	renal outer medullary potassium channel
RRT	renal replacement therapy
SOP	standard operating procedure
SST2	somatostatin receptor 2
TAL	thick ascending limb
TIM-1	immunoglobulin and mucin-containing molecule 1 family
TKV	total kidney volume
TRPP2	transient receptor potential channel 2
UTI	urinary tract infection

Autosomal Dominant Polycystic Kidney Disease

Clinical Description

Autosomal dominant polycystic kidney disease (ADPKD) is the most common inherited kidney disease, affecting approximately 1 in 400 to 1 in 1,000 live births. ADPKD occurs in all races with an age-adjusted sex ratio towards male of 1.2 to 1.3.[1] The continuous development and progression of multiple bilateral renal cysts over decades destroy functional renal parenchyma and ultimately lead to a loss of kidney function. The epithelial cells of the cysts are highly proliferative and apoptotic, and show planar polarity defects and secretory behaviour.[2] In ADPKD, end stage renal disease (ESRD) takes place in 50% of affected subjects between their 50s and 60s with the need for renal replacement therapy (RRT). The systemic character of ADPKD implicates renal as well as extra renal manifestations, like hypertension, hepatic cysts, intracranial aneurysm and pain, collectively impairing quality of life (QoL).[1,3]

The diagnosis of ADPKD is clinically performed via ultrasound and family history. Family history is of help to confirm the diagnosis of ADPKD. However, positive family history may be absent in up to 40% of patients, attributable to undiscovered ADPKD in patients' first grade relatives.[4] Age specific criteria for ultrasound diagnosis are applied to identify patients at risk for PKD1.[5] Genetic testing will confirm diagnosis with distinction of PKD1 and PKD2 mutation.

Genetics

Two genes are causative for ADPKD. *PKD1*, located on chromosome 16p13.3, accounts for 85% of all cases. The remaining 15% are caused by mutations in the *PKD2* gene, located at chromosome 4q21. Approximately 1% of all ADPKD cases are neither linked to either *PKD1* nor *PKD2* mutations, suggesting the involvement of at least one, so far unindentified other gene.[6] De novo mutations account for 2 to 5% of ADPKD cases.[7]

Polycystin 1 (PC1), a large complex cell surface glycoprotein is encoded by *PKD1*. PC1 contains 4,300 amino acids and a large extracellular domain that supports protein-carbohydrate and protein-protein interactions. The structure of PC1 with its 11 transmembrane domains and short intracytoplasmic C-terminus contains a G-protein coupled domain and a coiled-coil domain, for interacting as receptor and mediating cell-cell and cell-matrix interactions. The *PKD1* gene includes 46 exons with a 52 kb genomic segment.[2] The function of PC1 is not fully elucidated to date.

PKD2, with its 69 kb genomic region and 15 exons, encodes polycystin 2 (PC2), which is involved in cellular calcium signalling.[6,7] The 968 amino acid containing protein PC2, also named transient receptor potential channel 2 (TRPP2), contains a cilium-targeting motif in the N-terminus. The C-terminus is involved in protein-protein interactions containing a calcium-binding motif and a coiled-coil domain.

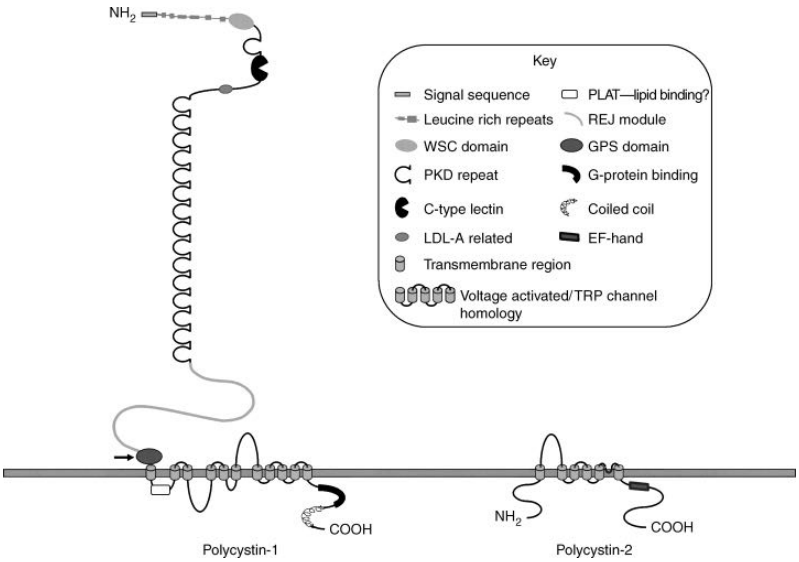

Figure 1: Predicted structure and topology of polycystin 1 and polycystin 2.[8]

The activity of PC2 is regulated by PC1.[2] PC1 and PC2 form a heteromeric complex by their C-termini at the cell surface of the primary cilium, acting as flow sensor in the primary cilium. Beside the cilium, PC1 and PC2 are localized in the plasma membrane and in the endoplasmatic reticulum. Decreased expression of PC1 and PC2 results in increased cell proliferation and apoptosis, disturbed planar cell polarity and extracellular matrix alterations. Furthermore, a change in salt and fluid transport from a reabsorptive to a secretory pattern occurs.[2,8,9] The genotype is reflected in phenotypic heterogeneity in ADPKD. *PKD1* mutations are associated with a more severe disease course with reaching ESRD approximately 20 years earlier compared with PKD2 mutations.[1]

Progress

In absence of family history, ADPKD often remains unrecognized until the appearance of clinically evident symptoms. Cyst growth is already seen in foetusses.[10] Early ADPKD symptoms occur in 2 to 5% of new-borns and can be mistaken for ARPKD.[4,11] A substantial intra-familial

variability of ESRD onset within each genotype adverts to the influence of environmental factors, epigenetic mechanism, and other modifying genes in the pathogenesis of ADPKD.[12] The disease commonly becomes clinical evident in the 3rd decade of life.

Renal Manifestation

The exponential increase of total kidney volume is determined by continuous cyst growth. The annual average kidney growth in early disease is 5.3% ± 4.0% with an estimated doubling of kidney volume over 8 years.[13] This increase in kidney volume is quantifiable in small periods of 6 month.[14] Cysts originate from all parts of the nephron, but only about 1% of the nephrons develop cysts.[15] Due to the capacity of the remaining nephrons to hyperfiltrate, kidney function stays stable over decades. Once the majority of renal parenchyma has been replaced by cystic tissue, kidney function declines. According to estimated glomerular filtration rate (eGFR), stages

Table 1: Stages of CKD by estimated glomerular filtration rate. Adapted from[16]

CKD stages	Description	GFR [ml/min/1.73m^2]
1	Normal to high	≥90
2	Mildly decreased	60 –89
3a	Mildly to moderately decreased	45 – 59
3b	Moderately to severely decreased	30 – 44
4	Severely decreased	15 – 29
5	Kidney failure	<15

Hypertension, blood pressure above 140/90 mmHg, is a common comorbidity in 75 to 85% of ADPKD patients. It is a major contributor to cardiovascular mortality and associated with faster progression to ESRD. Increasing evidence points to the activation of the renin-angiotensin-aldosteron-system (RAAS) being responsible for the pathophysiology of hypertension.[17] The

stretch and compression of the vasculature by cysts cause relative ischemia, probably leading to increased renin release.[1] Untreated hypertension is a major contributor to cardiovascular disease and is a major cause of death in ADPKD.

Nephrolithiasis, urinary tract infections (UTI) and gross hematuria are common and frequent renal morbidities in the course of ADPKD.[18-20] 16 to 25% of ADPKD patients experience at least one episode of kidney stones, due to metabolic abnormalities or stasis by virtue of increased pressure on collecting ducts caused by multiple cysts.[18,21]

60% of adult ADPKD patients report pain which is associated with the aforementioned co-morbidities.[1] The expansion of cysts and the compression of nearby tissue in kidney and liver are causes for pain, beside increased lumbar lordosis, traction of the renal pedicle and exaggerated pelvic tilt.[22] Chronically pain impairs patients quality of life (QoL). Predictors for accelerated disease progression are genotype, hypertension, hematuria, urinary sodium excretion, and urine concentration impairment.[10]

Extrarenal Manifestation

Hepatic cysts are a common extrarenal manifestation in ADPKD and more pronounced in female.[23] Associated with both genotypes, liver cysts are usually not impairing liver function. The excessive proliferation and dilatation of biliary ducts and peribiliary glands cause dispnea, create an early sense of satiety or cause oesophageal reflux thereby further impairing patients QoL. The impact on patients QoL justifies interventions to retard liver expansion.[24] In contrast to the kidney, cysts are not fully accounting for hepatomegaly. Increased liver parenchymal volume of ADPKD patients compared with healthy volunteers, hyperplasia and hypertrophy of hepatocytes have been investigated in mice with reduced PKD1 expression.[25]

Echocardiography reveals valvular abnormalities in 25% of the patients.[1] Left ventricular hypertrophy (LVH) occurs early and may include traditional risk factors like hypertension but also disease specific factors like fibroblast growth factor 23 (FGF23) but the causative mechanisms are incompletely understood.[17,26,27] The prevalence of intracranial aneurysms with potential severe outcome is higher in patients with ADPKD compared with healthy controls (5.8% versus 2.8%).[21] Supposing a positive family history, patients are recommended to undergo an angiographic screening per decade.[28]

Extra renal manifestations further include prostate cysts, sperm abnormalities, umbilical hernias, pancreatic cysts and colic diverticulosis and others.[29-32] Advanced kidney disease is associated with a severe dysregulation of endocrine, metabolic and hematological systems. Dysregulation of mineral metabolism presents as aberrations of calcium, phosphate, parathyroid hormone, FGF23, klotho and vitamin D metabolism.[33]

Treatment

Standard Care

Until recently no disease modifying treatment was available. The vasopressin 2 receptor antagonist Tolvaptan has been recently approved in Japan, Canada and Europe. This will be the first medication for the treatment of ADPKD. So far patients are treated according to standard medical care with the focus on comorbidities of ADPKD.

Early onset of hypertension may lead to an earlier onset of ESRD and blood pressure management should begin early in disease course.[34] A rigorous blood pressure control (95/60 to 110/75 mmHg) significantly decreases kidney enlargement but did not affect kidney function as shown in the HALT-PKD trial.[17]

Increased water intake reduces the levels of AVP and causes lower urine osmolality. Lower levels of AVP result in lower renal 3'-5'-cyclic adenosine monophosphate (cAMP) levels, which ameliorates disease progression through reducing fluid secretion and mitogenesis in cysts.[10,35]

There are several lifestyle recommendation that are mainly opinion-based.[36] It is recommended to restrict or eliminate the consumption of caffeine containing beverages and foods.[37] Methylxanthines like caffeine inhibit phosphodiesterases that break down cAMP and therefore intracellular basal levels of cAMP in renal epithelial cells increase.[38]

Once reaching ESRD, patients need to undergo renal replacement therapy (RRT), transplantation or dialysis.

New Therapeutic Targets

During the last three decades increasing knowledge about the basic pathophysiological processes of ADPKD defined novel therapeutic targets. Strategies for new therapeutic treatments focus on the inhibition of cell proliferation and fluid secretion into cysts as well as the reduction of intracellular cAMP.

Tolvaptan

The antidiuretic hormone arginine vasopressin and its secondary messenger cAMP promote cystic cell proliferation and fluid secretion, as shown in animal models of PKD.[39-43] Abnormal high renal cAMP levels in animal models may be found during the activation of the vasopressin receptor pathway. cAMP plays a major role in cystogenesis. The polycystin pathway, which is disrupted in ADPKD, is involved in regulation of intracellular calcium. The disruption

of the polycystin pathway results in a decreased release of Ca^{2+} from intracellular stores of renal epithelial cells. This stimulates adenylyl cyclase VI and inhibits cAMP-dependent phosphodiesterases, resulting in increased levels of intracellular cAMP.[39]

Tolvaptan is a selective vasopressin 2 receptor antagonist and was originally approved for the therapy of the syndrome of inappropriate antidiuretic hormone secretion (SIADH) and cardiac disease induced edema.[44] The TEMPO 3:4 trail in ADPKD patients with moderately reduced eGFR $\geq$ 60 ml per min per 1.73m^2 and a kidney volume $\geq$ 750 cm^3 showed a reduced annual growth rate under tolvaptan treatment compared with placebo group (2.8% versus 5.5% per year).[45]

mTOR Inhibitors

Inhibitors of the mammalian target of rapamycin (mTOR) block cystogenesis in animal models of PKD.[46,47] The Ser/Thr kinase mTOR is involved in the regulation of cell proliferation, cell growth and cell cycle progression.[46,48] The ribosomal protein S6 kinase is a downstream target of the mTOR complex, composed of mTORC1 and mTORC2, and regulates translational initiation of cell proliferation and growth.[48] Increased levels of S6 kinase and activation of the mTOR pathway has been found in animal models of PKD.[46]

The mTOR inhibitor Sirolimus has strong anti-proliferate properties and is clinically used to prevent organ rejection following renal transplantation.[49] Despite the promising results from preclinical studies, mTOR inhibition with Sirolimus and Everolimus failed to show an effect on kidney function and disease progression in APDKD patients.[50,51]

Long Acting Somatostatin Analogue

The effect of somatostatins to reduce renal and hepatic volume in ADPKD has been found by chance during the treatment for neuroendocrine tumors. The biological somatostatin peptide is secreted by pancreatic islets, the gastrointestinal tract, nervous system and thyroid gland. Its receptor type 2 (SST2) is expressed in liver and kidney, where it regulates cAMP levels, thereby influencing cell growth and proliferation. The treatment with the long acting somatostatin analogues Octreotid and Lanreotid showed in three studies with small groups of patients with ADPKD and polycystic liver disease (PLD) decelerated liver growth rates in patients with ADPKD and polycystic liver disease.[52-54] In these studies liver volume decreased only by 3 to 5% with an initial liver volume of 4,400 to 5,500 cm^3. GFR was not affected by this treatment. The investigators of the multi-centre single-blind randomized ALADIN trial demonstrated a significant reduction of annual kidney growth rate in 38 ADPKD patients treated with long acting somatostatin analogues compared with placebo at one year of treatment. Unfortunately, growth rate was similar in both groups at three year follow up.[52] Still, adequately powered studies are needed to assess the efficacy of somatostatin analogues for ADPKD.[44]

Preclinical studies

Several substances have been tested in preclinical studies to retard PKD progression. Roscovitine, a cycline dependent kinase inhibitor, showed promising results in inhibition of cystogenesis and improvement of renal function in animal models.[55] Inhibitors of the cystic fibrosis transmembrane conductance regulator (CFTR) retarded cyst growth in a PKD animal model. TRAM-34, an intermediate-conductance inhibitor of Ca^{2+}-activated K^+-channel, inhibits cyst growth in ADPKD cells.[56,57]

Need for observational studies

The natural course of ADPKD with strong inter- and intrafamilal onset of ESRD and the long clinically silent phase make it difficult to identify patients that will benefit most from upcoming therapies. Increasing basic knowledge of ADPKD has led to the identification of multiple novel targets in pre-clinical studies as described before. Once, these substances become available, the question has to be answered which patients to select for treatment. The identification of surrogate markers to assess disease severity and risk of progression and to monitor the effect of interventions on the course of disease remains an important goal and is an unmet medical need. The nature of observational studies to observe and to follow the patients over a long period makes it possible to evaluate markers of early disease progression and to identify patients with faster disease progression. Fast progressing patients at early disease stage will benefit most from a medical intervention. Observational studies, like CRISP and EuroCYST in ADPKD are extremely valuable and will add important results for the understanding of the pathogenesis and characteristics of ADPKD.[13,58]

Biomarkers

Functional markers versus markers of disease

The National Institutes of Health Definition Working Group defines a biomarker as an objectively measurable indicator of normal biological and/or pathological processes, or pharmacological response to a therapeutic intervention.[59,60] Biomarkers help to define disease states in a certain condition, to assess prognosis, to predict risk, and to quantify the effect of a pharmacological approach (figure 2). Thereby they can be of biochemical origin and are measurable in biological media such as human tissue, cells or fluid.[61] Type 0 biomarkers reflect the natural history of a disease correlating with clinical indices whereas biomarkers of type 1 capture the effect of an intervention.[62] In clinical ADPKD studies the effect of an intervention or the assessment of patients at risk is reflected most valuable with clinical end points. The achievement of hard endpoints, like death, stroke or ESRD in clinical studies is a time and cost intensive endeavour and often not practical. Results may be delivered with delay for the study population of impending need.[61] Therefore substitutes for clinical endpoints are used to assess the effect of an intervention or to assess patients at risk for disease progression. These surrogate markers are surrogates for impracticable clinically meaningful endpoints.[59]

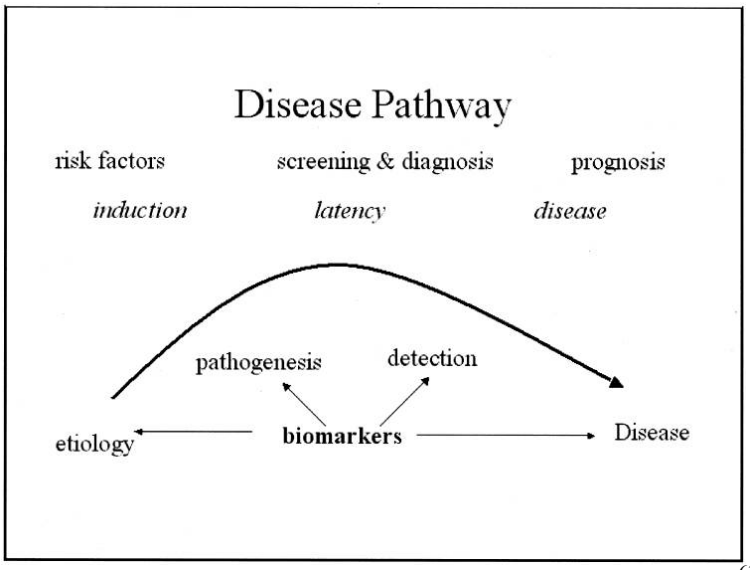

Figure 2: Disease pathway and potential impact of biomarkers.[63]

A major concern in the application of biomarkers is the inter- and intraindividual variability. To monitor patients with a chronic disease longitudinally tight intraindividual variability of the biomarker is crucial. In contrast, group variability is a desired outcome in studies. Considering possible variations in studies can prevent misclassification of the patients. Validity and reliability are crucial for obtaining precise results.[63]

Biomarkers in ADPKD

Biomarkers of disease identify patients at risk, reflect the natural history of the disease and can be targets for clinical trials. The interest in reliable biomarkers in the field of nephrology is increasing and many potential markers have been studied in conditions, like CKD and acute kidney injury (AKI).[64] In ADPKD, biomarkers should identify and quantify pathological processes and should reflect specific renal glomerular and tubular regions. The extent of renal damage can be assessed by markers derived from blood, urine and biopsy specimens as well as from imaging procedures and DNA analysis. The advantage of urine as specimen for assessment

of biomarkers is its easy accessibility, with preferably low burden for the patient. Furthermore, urine is relatively thermodynamically stable, allowing easier handling during analysis.[65] In the following section different biomarkers of importance in ADPKD will be discussed.

Hematuria

Early clinical symptoms include symptomatic or asymptomatic hematuria in children and adults affected with ADPKD.[10,66,67] In CKD, hematuria is commonly of glomerular origin, caused by defects of the glomerular basement membrane, whereas hematuria in ADPKD is of tubular origin.[68] Macrohematuria is the visible presence of red blood cells in urine and occurs in 30 to 50% of ADPKD patients. In these patients, macrohematuria occurs frequently and 25% of ADPKD patients report more than six aematuric episodes during disease course.[69,70]

The underlying causes for this co-morbidity are cyst hemorrhages into the renal pelvis, the rupture of cyst lining vessels due to overactive vascular endothelial growth factor resulting in enhanced angiogenesis in the kidney.[10,71] Further reasons are UTI, nephrolithiasis, glomerulonephritis, artiovenous fistula, trauma and tumors.[72] In clinical routine the absence of a benign cause and infection distinguish between glomerular and non-glomerular cause. Hematuria is a risk factor for an accelerated disease progression in ADPKD and episodes of macrohematuria are associated with increased renal volume and hypertension.[13,69,73-76] Early and recurrent episodes are associated with more severe disease course and worse renal survival.[9,71,77]

Microscopic hematuria is rarely reported in ADPKD and often asymptomatic. The cut off for microscopic evaluation differs between laboratories and is between more than 2 to more than 5 erythrocytes per high power field. The absence of routine assessment makes microhematuria often an incidental finding.[78] The prevalence of microhematuria in the general population ranges between 0.2 and 21%, depending on age and sex, follow up time and number of participants in

the study population.[79] A large study in young CKD patients in Israel revealed persistent microhematuria in 0.3% during a 22 years follow-up. Of those, 0.7% developed ESRD compared with 0.04% of CKD patients without persistent microhematuria.[80] Hemoglobin from hematuria episodes in the tubular system may by itself be a risk factor for disease progression. Free hemoglobin in the tubular system promotes reactive oxygen species and may induce renal ischemia. Uptake of hemoglobin into the tubular cell increases hydroxyl radicals, apoptosis and secretion of cytokines, contributing to inflammatory responses.[68]

Albuminuria

Albuminuria is a well-known marker for glomerular permeability and a predictor for kidney function decline. Urinary excretion of albumin is associated with CKD progression, decreasing eGFR, myocardial infarction and mortality.[81-84] The advantage of albuminuria as valuable marker is that its urinary appearance preceedes a change of other established markers of kidney function, like creatinine. It is produced and secreted by hepatocytes and is present in the plasma of all mammals**.** As the most abundant plasma protein it represents 60% of the total plasma protein. The urinary excretion in health is below 30 mg per 24 h. An excretion of 30 to 300 mg albumin per 24 h is classified as microalbuminuria. Macroalbuminuria reflects an excretion of 300 mg to 3 g albumin per day. Nephritic range albuminuria describes urinary excretion above 3 g per 24h.[81] One important function of the 69 kDa protein is the maintenance of oncotic pressure between plasma and interstitial space.[85] Furthermore it is a transport protein for endogenous and exogenous molecules (amino acids, fatty acids, hormones, metabolites, pro-inflammatory substances, drugs etc.).[86,87] Thus a loss of albumin and its ligands via urine should be prevented. The radius and negative charge of albumin restrict filtration in the Bowmans' capsule. Hence, the fractional excretion of albumin is about 0.05 to 0.1%.[85] Quantitatively

reabsorption of albumin in the proximal convoluted tubule takes place via absorptive receptor-mediated endocytosis. Thus, albuminuria can occur from glomerular or tubular processes.[86]

Albuminuria is common in CKD and usually preceedes the fall in GFR. Increased excretion of albumin in ADKD is associated with TKV, renal blood flow and GFR, myocardial infarction and mortality.[81-83,88,89] Steeper declines in kidney function are associated with higher levels of proteinuria.[34] Albuminuria is also observed in children with postnatal diagnosis of ADPKD.[90] The assessment of microalbuminuria is an accurate method to detect patients at risk for cardiovascular morbiditiy.[91] The albumin-to-creatinine ratio was added to the CKD stages by the Kidney Disease: Improving Global Outcomes (KDIGO) in 2012.[92]

Neutrophil gelatinase-associated lipocalin-1 (NGAL)

The 25 kDa protein NGAL is expressed in kidney, liver, stomach, trachea, colon, lung, salivary gland, and epithelial and vascular cells.[82,93,94] As a member of the lipocalin superfamily, NGAL forms calyces to bind low molecular weight molecules, like bacterial siderophores.[95] NGAL can either eliminate iron or deliver it intracellularly for iron-depending enzymes. Holo-NGAL facilitates the cytoplasmic iron supply by binding and transporting iron particles into the cells, where it activates iron-dependent pathways through the release of its siderophore-iron complex in the cytoplasmic region.[96] In contrast, internalized Apo-NGAL carries iron to the extracellular space, leading to a depletion of intracellular iron.[97] The cell activity of NGAL is mediated by the brain type cation transporter 24p3R and the megalin multiscavenger complex. In the presence of NGAL the enterocholin-iron-complex of gram-negative bacteria binds to it, thereby preventing iron transfer to bacteria. NGAL deficient mice are more susceptible to infections with gram-negative bacteria compared to wildtype mice.[98]

NGAL is almost completely reabsorbed in the proximal tubule by endocytosis and under physiological conditions only small amounts of NGAL are excreted via urine.[97] During renal injury NGAL expression is increased in renal distal epithelial cells and urinary excretion rises before creatinine rise is observed.[99] Elevated levels of NGAL are predictors for disease progression and have been reported in AKI and CKD.[89,97,100,101] Levels are massively increased following nephrotoxic and ischemic defects in the proximal and distal tubules and can be measured very sensitively in blood and urine.[82]

Rising urinary excretion of NGAL is associated with TKV, kidney function worsening within one year and accelerated progression to ESRD in ADPKD.[82,89,99,101,102] Furthermore, the number and size of cysts was positively associated with urinary NGAL, independently of renal function.[103]

Kidney Injury Molecule-1 (KIM-1)

Under physiological conditions the kidney injury molecule 1 (KIM-1) is absent from human urine. It was discovered while studying molecules involved in AKI and it is the founding member of the immunoglobulin and mucin-containing molecule 1 family (TIM-1). It is exclusively expressed in the cilium of proximal tubular cells.[104] Secretion takes place during proliferation and dedifferentiation and the 90 kDa ectodomain is cleaved into urine under the control of MAP kinases.[105] The MAP kinase-signalling pathway is activated by cell stress. KIM-1 is a phosphatidylserine receptor, mediating phagocytosis of necrotic cells by acting as apoptotic factor.[93,105] It facilitates repair by removing exosomes and dying cells in the proximal tubular cell. Immunological studies show that ligand concentration determines a stimulatory or inhibitory effect on KIM-1.[105] So, the KIM-1 receptor seems to fulfil two different functions: first its role in repair processes in the proximal tubular cell and second, its action in inflammation by attracting

macrophages and fibroblasts to injured epithelial cells.[106,107] The upregulation of KIM-1 has been observed in many states of kidney injury and chronically KIM-1 expression leads to chronic kidney failure.[108]

Since KIM-1 is located at the cilium it may interact with TRPP2 and therefore play a role in ADPKD. In fact, KIM-1 expression was found in subsets of cysts of the PKD1 mouse model, whereas wildtype mice did not express KIM-1 in the kidney.[109,110] The expression was associated with a loss of polarity, decreasing complexity and decreased levels of the Na-K-ATPase. KIM-1 is associated with the N-terminus of TRPP2 but fails to interact in PKD. Also, mutations in KIM-1, lacking Y350, may interrupt the flow induced calcium increase. So far, knockdown of KIM-1 in epithelial tubule cells (MDCK) was not sufficient to prove the effect on calcium flow.[104]

Urinary KIM-1 is increased in patients with AKI, CKD and is associated with renal graft loss and ESRD.[89,93,111-114] Even so studies investigating KIM-1 in ADPKD are meagre, urinary KIM-1 excretion seems to be increased during the course of ADPKD. A positive association of KIM-1 levels in 24 hour urinary samples with TKV, independent of albuminuria in ADPKD was reported by Meijer et al.[89] Also Kühn et al. reported an increase in KIM-1 levels in PKD patients.[110]

Uromodulin (Tamm-Horsfall Protein)

Uromodulin (Tamm-Horsfall Protein) is the most abundant protein in human urine and a major component of urinary casts. The daily excretion is about 20 to 100 mg per day in healthy persons, depending on diet, urine volume and physical activity.[115-118] The glycosyl-phosphatidylinositol (GPI) anchored protein is exclusively produced in and secreted via the apical plasma membrane of the epithelial cells of the thick ascending limb (TAL) of the Loop of Henle, a tubule segment with high electrolyte permeability. Uromodulin is also localized in cells

of the early distal convoluted tubule.[118] It is released into urine by proteolytic cleavage from the luminal surface of the TAL, by yet unknown proteases.[119,120] The 80 to 90 kDa protein contains 616 amino acids.[115,116,121] The clear physiological roles of uromodulin are still elusive. Evidence suggests that uromodulin prevents UTI and nephrolithiasis.[122] Uromodulin averts UTI by binding to uropathogenic Escherichia coli via its 8 sites of N-glycosylation. The preventive activity of nephrolithiasis is attributable to prevention of calcium oxalate crystal aggregation through its epidermal growth factor domains with calcium-binding domains. Uromodulin-KO mice with absent uromodulin expression and excretion are more susceptible to UTI due to binding of uropathogenic E.coli, preventing the binding to uroplakin receptors of the urothelium surface.[123,124] Mutations in the *UMOD* gene, located on chromosome 16, cause lower levels of urinary uromodulin excretion in a gender- and sex-independent manner and independent of severity of renal failure. Interestingly, subjects with decreased uromodulin excretion do not present UTI or nephrolithiasis more frequently compared with healthy.[116] This may be explained by the fact that humans with *UMOD* mutation still excrete small amounts of uromodulin via urine whereas uromodulin KO mice did not secrete uromodulin at all.[123] Also in familial juvenile hyperucemic nephropathy (FJHN) the urinary excretion of uromodulin is diminished. In contrast to decreased urinary expression in this disease setting the expression is increased and mutated uromodulin accumulates in the renal tubules.[125] There is increasing evidence that the protein regulates the sodium-potassium-chloride transporter (NKCC2) and the renal outer medullary potassium channel (ROMK) in the TAL.[122] As shown in experiments, ROMK2 channel delivery to the plasma membrane is increased by uromodulin expression. The role of uromodulin in innate immunity has been deduced from its interaction with parts of the immune system as response to nephron damaging conditions.[115]

The current scientific state of knowledge reveals a decreasing urinary uromodulin excretion and is correlated with eGFR in different conditions of kidney disease, including ADPKD.[119] Decreasing levels of uromodulin in urine of CKD patients, not presenting *UMOD* mutation, may be caused by a reduced number of uromodulin secreting cells attributable to tubule damage.[118,126,127] This evidence suggested to study the excretion of of uromodulin as biomarker for CKD. Urinary uromodulin levels in ADPKD patients have been investigated for the first time by us.

Clara Cell Protein 16 (CC16)

Tubular markers reflect the secretory and reabsorptive dysfunction of the tubule system of the kidney. Traditionally, analysis of cumulative proteinuria gives broad information predominantly about the glomerular state. Changes in specific tubular parts are of high predictive value. Low molecular weight proteins (LMWP) (13 to 33 kDa), like CC16, display a broad functional spectrum.[128-130]

The homodimeric CC16 protein was first isolated by Dakopatts in the 1980s and is predominantly expressed in human non-ciliated bronchial Clara cells and to a lower extent in nose mucosal epithelial cells, endometrium and in foetal kidney.[131,132] Its expression in the lung is induced during cell differentiation and diminished by toxic pulmonary agents.[133] Secretion into the air spaces of the respiratory tract is followed by passive diffusion into plasma. The 15.8 kDa protein has been studied in inflammatory diseases, where serum CC16 serves as a marker for pulmonary damage. Animal and human studies report increased serum CC16 levels following exposure to pulmonary toxicants, like cigarette smoke. Still, its precise physiological function is not clear. It is involved in bronchial tissue repair and protection from respiratory tract inflammation, by inhibiting phospholipase A, the enzyme responsible for production of

prostaglandins and leukotrienes, and for interferon (IFN)-γ production and activity.[132] The high interindividual, diurnal and gender variability of human serum CC16 remain unexplained so far. Postrenal secretion from prostate decreases its specificity in men.[129,134]

CC16 is freely filtered in the glomerulus, reabsorbed in the proximal tubule and excreted only in small amounts in urine under physiological conditions.[129,130,134] The reabsorption in the proximal tubule will likely take place via cubilin-megalin mediated endocytosis by binding of CC16 to cubilin, as indicated by animal studies.[133,135] As kidney function decreases, urinary CC16 excretion increases. Impaired tubular integrity cause elevated urinary excretion of CC16, possibly by destruction of the endocytosis machinery due to impaired function and morphology of the proximal tubule.[133]

The properties of CC16 would per se qualify as biomarker of proximal tubular injury, due to its stability in urine, its sensitivity and its anionic character. Urinary CC16 levels have so far not been reported in ADPKD and it needs to be evaluated whether or not CC16 is able to assess patients' disease state in ADPKD.

Imaging as a Marker

ADPKD is characterized by continuous kidney enlargement due to growth of cysts in medulla and cortex.[136] Cystogenesis starts early, is continuous and changes in TKV can be measured in short 6-month intervals.[14] TKV is associated with cyst growth and will be multiply the healthy volume of 150 ml during disease course.[137] As commonly observed volume increases equally in both kidneys despite heterogeneity in cyst size.[13] TKV is associated with pain, hypertension, gross hematuria, and proteinuria and is now accepted by researchers as prognostic biomarker and used as endpoint in clinical studies investigating new medical treatments in ADPKD.[138] Kidney volume is inversely correlated to eGFR. Kidney volume growths with an

annual growth rate of 5.3% ± 4.0% in early disease stage.[137] Height adjusted total kidney volume (htTKV) of 600 cm^3 per m predicts the progression to CKD stage 3 within 8 years.[139] Magnetic resonance imaging is the gold standard in imaging procedures and can precisely and reproducible detect small cysts without application of contrast media.[138] Still, kidney imaging using MR-techniques are not in clinical routine in all European countries and not available to all patients. Imaging and volume quantification requires expertise, is technically demanding and expensive. The quality of the images relies on patient complicance and on specific parameters, like sequences and slice thickness.[139] Additionally, volume quantification using stereological approaches is time consuming, since it has to be done manually, tracing each slide, which are usually 30 to 40 slices with a thickness of 3 mm per slice, of an MRI to evaluate total kidney volume. New approaches like the mid-slice area measurement seem to be precise to estimate total kidney volume in clinical setting while saving time.[140]

Hypothesis and Aims

During the last decade, much knowledge has been gained about the pathophysiological processes in ADPKD. Different medical treatments have been investigated in preclinical and in clinical studies.[35,46,50-52] Until recently, no medication for treatment of ADPKD was available in Europe and clinical practice concentrates on the treatment of co-morbidities aiming to slow down disease progression. Specific characteristics of the disease, like the strong inter- and intrafamilar variability of disease course remains unexplained as well as the difficulty to assess disease state and prognosis.

The aim of the EuroCYST Initiative is to contribute to supply the aforementioned unmet needs in ADPKD. The design of the study has the potential to identify progression factors and biomarkers, and to assess disease stage-specific mortality, morbidity and health-care costs as well as Quality of Life. The development and implementation of this large observational international study will include 1,100 patients in 13 centres in 10 European countries. This approach is so far unique in the field of ADPKD and will harmonize the fragmented research in Europe.

The accurate assessment of disease state and progression probability needs easy obtainable and reliable biomarker. Kidney function markers like serum creatinine and estimated glomerular filtration rate (eGFR) remain stable for long time and their ability to accurately assess disease state and to predict progression in the early course of ADPKD is limited. We hypothesized that ADPKD patients excrete a specific pattern of urinary proteins that may serve as biomarkers to assess disease state and predict disease progression in early disease state. Possible markers were evaluated from urine specimens of ADPKD patients to meet the requirements of evaluating biomarkers with convenient accessibility and low burden for patients.

First Author Manuscripts

Building a network of ADPKD reference centres across Europe: the EuroCYST initiative

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Contribution to this manuscript:

The study protocol has been compiled and written by Katja Petzold. The study processes have been defined and implemented by Katja Petzold in collaboration with the management team. The data management system, including all electronical case report forms, hase been designed and programmed and implemented by Katja Petzold. Katja Petzold coordinated the study, including the initiation of study centres. The manuscript was written and the figures were made by Katja Petzold.

Abstract

Background. Autosomal dominant polycystic kidney disease (ADPKD) is the most common monogenic inherited kidney disease, affecting an estimated 600,000 individuals in Europe. The disease is characterized by age-dependent development of a multiple cysts in the kidneys, ultimately leading to end-stage renal failure and the need of renal replacement therapy in the majority of patients, typically by the fifth or sixth decade of life. The variable disease course, even within the same family, remains largely unexplained. Similarly, assessing disease severity and prognosis in an individual with ADPKD remains difficult. Epidemiological studies are limited due to the fragmentation of ADPKD research in Europe.

Methods. The EuroCYST initiative aims: (i) to harmonize and develop common standards for ADPKD research by starting a collaborative effort to build a network of ADPKD reference centres across Europe and (ii) to establish a multicentric observational cohort of ADPKD patients. This cohort will be used to study factors influencing the rate of disease progression, disease modifiers, disease stage-specific morbidity and mortality, health economic issues and to identify predictive disease progression markers. Overall, 1100 patients will be enrolled in 14 study sites across Europe. Patients will be prospectively followed for at least 3 years. Eligible patients will not have participated in a pharmaceutical clinical trial 1 year before enrolment, have clinically proven ADPKD, an estimated glomerular filtration rate (eGFR) of 30 mL/min/1.73 m^2 and above, and be able to provide written informed consent. The baseline visit will include a physical examination and collection of blood, urine and DNA for biomarker and genetic studies. In addition, all participants will be asked to complete questionnaires detailing self-reported health status, quality of life, socioeconomic status, health-care use and reproductive planning. All subjects will undergo annual follow-up. A magnetic resonance imaging (MRI) scan will be

carried out at baseline, and patients are encouraged to undergo a second MRI at 3-year follow-up for qualitative and quantitative kidney and liver assessments.

Conclusions. The ADPKD reference centre network across Europe and the observational cohort study will enable European ADPKD researchers to gain insights into the natural history, heterogeneity and associated complications of the disease as well as how it affects the lives of patients across Europe.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common monogenic genetic diseases, affecting ~1 in 1000 live births, that is ~600 000 individuals in Europe.[1] The disease is characterized by the development of multiple cysts in both kidneys and by potentially serious complications.[136] Disease manifestations impairing quality of life include hypertension, chronic pain, intracranial aneurysms, abdominal hernias, hematuria, urinary tract infection and kidney stones. Kidney function is often preserved up to the age of 40, but subsequently glomerular filtration rate (GFR) decreases and often leads to end-stage renal disease (ESRD).[141] However, there is wide variability between subjects in disease course, even within families that share the same mutation, with some patients never reaching ESRD.

ADPKD represents a major burden for public health in the EU, estimated at €1.6 billion annually for direct medical costs related to renal replacement therapy.[142] This figure is an underestimate of the true economic burden, because it does not take into account costs related to co-morbidities that frequently occur in patients with impaired kidney function and the loss of income generation that is often observed in subjects with later stage kidney disease. Research on

prevention of ADPKD-related complications could therefore offer a tremendous return on investment.

At present, there are no approved disease-modifying treatments available for ADPKD. Intensive basic research during the last three decades has contributed to a clearer understanding of the basic pathophysiological processes that lead to renal cyst formation in subjects affected by ADPKD with definition of novel therapeutic targets.[12,143] In animal studies, some of the treatments directed at these targets, such as mammalian target of rapamycin (mTOR) inhibitors, somatostatin analogues and vasopressin V2-receptor antagonists (V2RA), have shown promising results.[39,49,144] The clinical trials testing mTOR inhibitors showed no clear impact on disease progression.[48,50,51] However, recent results from the TEMPO 3:4 and ALADIN trials with the V2RA tolvaptan and the somatostatin analogue octreotide have shown an effect on the rates of kidney growth and kidney function decline, although long-term treatment safety needs to be addressed.[35,52] If these therapies become available for clinical use, the pivotal question will be which patients to select for treatment. One can hypothesize that ADPKD patients with rapid disease progression would benefit most and that treatment should be started at an early stage of disease, when the kidneys are more likely to respond to an intervention. Since ADPKD is characterized by a long period of stable kidney function, due to compensatory filtration of unaffected nephrons, kidney function does not accurately reflect disease severity nor prognosis.[13] Genotype (PKD1 as opposed to PKD2 mutation), male gender and young age at onset of hypertension among others associate with faster disease progression in ADPKD.[145,146] However, the predictive value of these variables is limited and untested in large prospective cohorts. The identification of surrogate markers to assess disease severity and risk of progression and to monitor the effect of interventions on the course of disease remains an important goal and is an unmet medical need. Results of smaller observational studies in ADPKD cohorts, such as CRISP

and SUISSE ADPKD, suggest that changes in total kidney volume (TKV) are a predictor of subsequent loss of kidney function.[147] Magnetic resonance imaging (MRI) has a greater sensitivity for the detection of small cysts and allows to measure kidney and liver volume more precisely compared with ultrasound. It has been shown that MRI can already reliably detect changes in TKV that occur during 6 months of follow-up.[14] However, the two interventional trials with mTOR inhibitors (refs [50] and [51]) did not observe a correlation between total renal volume and disease progression (as measured by renal function) questioning whether TKV is a suitable surrogate marker for disease progression. In addition, this imaging technique is not routine clinical practice for ADPKD subjects in all European countries. Thus, there is a need to discover clinical factors or new biomarkers that predict the rate of disease progression.

Building a large, well-characterized cohort of ADPKD subjects who are followed in a longitudinal observational cohort study has the potential to identify progression factors and biomarkers, and to assess disease stage-specific mortality, morbidity and health-care costs. This knowledge should translate into new diagnostic and therapeutic modalities. This approach requires a coordinated multinational action within a network of ADPKD reference centres. The EuroCYST initiative aims to build such a network and to establish a large-scale pan-European ADPKD cohort serving as a versatile and powerful clinical research platform. Since EuroCYST is an academic initiative and not industry driven, free access to information and pseudo-anonymized biomaterial is ensured, as approved by a research oversight committee.

Methods

Objectives

The primary objectives of the EuroCYST initiative are to:

- (i) Build a network of ADPKD reference centres across Europe to provide a translational research platform that will enable EU researchers to study the pathogenesis, progression factors, morbidity, co-morbidity and health economic issues in ADPKD patients over a wide range of kidney function and kidney volume.
- (ii) Harmonize and develop common standards for ADPKD-related research by a collaborative effort to establish a pan-European ADPKD cohort.
- (iii) Harmonize and develop a common ADPKD biobank that includes standardized, quality-controlled biomaterials for translational research.
- (iv) Create a scaffold to facilitate the integration of current and upcoming technologies to ADPKD practice.
- (v) Develop evidence-based best practice and need assessments for ADPKD by utilizing the outcomes of the EuroCYST initiative and by engaging with relevant stakeholders, including patient organizations, clinical and research networks, legislators, policymakers and the pharmaceutical industry.
- (vi) Serve as an impetus to expand ADPKD training programmes at all levels by establishing collaborative and educational liaisons as well as provide standard criteria for effective management protocols in ADPKD.
- (vii) Improve awareness of the relevance of ADPKD including disease-specific complications and socioeconomic consequences of the disease among health-care professionals and payers.

EuroCYST strategy and organization

To build a cohort for a longitudinal observational study, 14 centres in 10 countries across Europe (Figure 1) will enrol 1100 adult ADPKD patients until the end of 2015. The study is funded by a grant of the ERA–EDTA with 1 million Euros. For optimal utilization of the funding, centres with expertise in ADPKD and already existing local clinical cohorts have been co-opted, so that efforts do not need to focus on recruitment, but can be invested into the establishment of the cohort infrastructure, uniform data recording and in-depth analysis of several time and thus resource-consuming aspects, such as assisted patient interviews using standardized questionnaires.

The enrolment phase started in summer 2013; within 1 year, 250 participants should be included. To reach the goal of recruiting 1100 ADPKD patients, each participating ADPKD centre will enrol at least 50 and up to a maximum of 100 patients within 2 years to ensure a representative distribution of patients across Europe. In a second step, which is beyond the current funding period, the cohort could be extended in four ways to: (i) prolong the intended duration of follow-up to longer than 3 years, (ii) increase the number of patients up to 5000 or more through the participation of additional European study centres with a minimum essential dataset, (iii) increase the information density per patient for specific aspects (e.g. cardiovascular pathology, imaging and genetics) or (iv) enrol partners, children and parents of index patients (three-generation cohort). The participation of additional centres will be possible if appropriate funding is obtained. Collaborations at various levels with other research groups to share data and biomaterials in order to achieve maximum scientific output will be encouraged. To this end, an open and transparent ancillary study policy has been established as part of the study protocol. A Steering Committee has been established and is meeting at least twice a year. All decisions by the

Steering Committee will be approved on a 75% majority. The establishment of a Central Study Coordination Team, which meets on a bi-weekly basis, will ensure rapid and successful project implementation and progress.

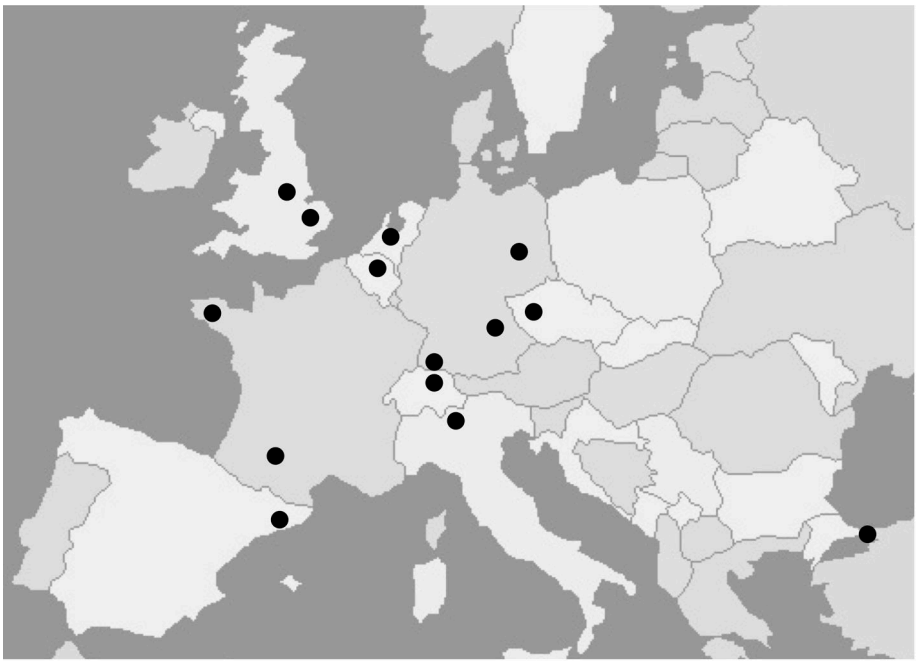

Figure 1: Location of participating study sites

Eligibility of cohort participants

Following a consecutive enrolment approach, all patients with ADPKD are considered as potentially eligible for the study at pre-screening and will undergo screening for the study. Patients aged 18 years and older with an estimated GFR (eGFR) of 30 mL/min/1.73 m^2 and higher [chronic kidney disease-epidemiology collaboration (CKD-EPI) formula], having a diagnosis of ADPKD established based on kidney ultrasound and family history (modified Ravine criteria) who have not taken part in a disease-modifying trial at least 1 year or shorter before enrolment and are able to provide written informed consent, will be eligible for enrolment into the EuroCYST cohort.[5,148] Table 1 displays the inclusion and exclusion criteria. Exclusion criteria include the likelihood of reaching ESRD within 1 year after enrolment or significant heart

disease according to New York Heart Association Stage IV (NYHA Stage IV). A stratification strategy based on subjects' eGFR will avoid a selection bias. Thus, 40 to 60% of included patients in each study centre need to have an eGFR of 60 mL/min/1.73 m^2 and more.

Study design

Patients who meet the inclusion criteria will be invited to participate. All potential participants will receive detailed information about the study both verbally and in writing. Local ethical committees will approve the study, and the protocol has to fulfil the local regulatory requirements and com- plies with Good Clinical Practice (GCP) Guidelines.

The study design is shown in Figure 2. At baseline, a detailed medical and ADPKD-specific assessment will be performed. Family history/pedigree information will be collected, as well as information on medical resource use (health-care visits, hospital admission, procedures and medication), productivity (employment and absenteeism), information culture within families and reproductive planning. The economic and social position, based on income, education and occupation, will be assessed. Quality of life will be measured by asking patients to complete the KDQOL-SF 1.2™ questionnaire, which includes questions relating to patients' general health, kidney disease and about the effect of the disease on activities of daily living. The protocol will also require a physical examination.

Follow-up visits will be conducted on an annual basis until the end of study, withdrawal, ESRD or death. Follow-up visits will include physical examination, laboratory analyses and completing the aforementioned ADPKD-related questionnaires. Participating patients will be treated according to current standards of care in routine clinical practice within each country.

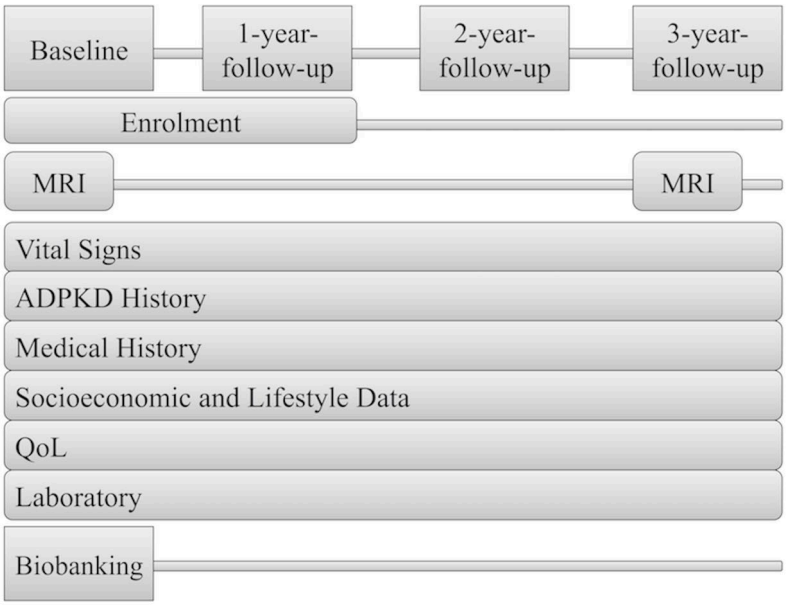

Figure 6: Study design

Magnetic resonance imaging

MRI will be used to measure the different magnitudes and volume parameters of kidney and liver. MRI will be performed at the baseline visit and is recommended at 3-year follow-up. To obtain high-quality renal and hepatic imaging and maintain consistency between the centres participating in the trial, a standardized protocol has been developed. The MRI acquisition protocol includes T2 single-shot fast/turbo spin-echo images with fat-saturation, FISP or FIESTA 3D spoiled gradient echo and T1-3D spoiled gradient echo. Images will be sent using the Picture Archiving and Communication System (PACS) to the centres in Zurich and Bergamo, where a read out by trained personnel will be performed. Read out of the scans includes whole kidney and cyst volume, length, depth, width of the kidney, numbers of cysts and also liver and liver cyst volume, using the workstation GE Advantage and the programme volume viewer.

Collection and storage of biomaterials

The collection of material for biobanking will be conducted at the baseline visit. A standard operating procedure (SOP) harmonizes the procedure for sampling, pre-processing and storage of biobanking material within the EuroCYST initiative. Serum, plasma and whole blood collected on EDTA and spotted, and a 24-h urine sample will be collected, processed and aliquoted. They will be shipped in batches to a central biobank storage facility where an automated −80°C sample library management system is in place to handle the de-identified 2D bar-coded sample vials. The whole blood tubes will be shipped to a central, certified genetic laboratory for DNA extraction and storage. The database will be kept separately with a secure method to link clinical information to biological samples.

Data management and protection

Data collection and data management will be conducted using the web-based data management system SecuTrial®, with a data capture, which has been approved by the US Food and Drug Administration (FDA) and that fulfils the requirements of the International Conference on Harmonization Good Clinical Practice (ICH GCP) and Good Clinical Data Management Practices (GCDMP). All electronic case report forms (eCRF) have been implemented into this system. Figure 3 shows the different subject areas of the data bank that are reflected in the eCRF. Data will be stored for at least 10 years after study termination and a daily back up will be performed. The study data are saved on a separate server at the University Hospital Zurich, where the clinical trial centre, provider of the data management system, is located. Server access is controlled physically and electronically. Each patient will be pseudo-anonymized in a reversible manner, and all data introduced into the data management system are coded. Subject identification will only be possible at the local study site. Access to the system will be role

specific and will only be possible with a unique user-ID and password. High data quality will be ensured by performing regular monitoring and reporting of entered data by the coordination centre in Zurich. All study site data entered in the eCRF will be checked for completeness and plausibility according to predefined rules to draw attention to missing data or errors. Certified personnel will monitor the participating centre annually. All patients' written informed consent forms and all study files will be checked for complete- ness, and remain at the individual sites. In 10% of locally enrolled patients, the source files and eCRFs will be checked for accuracy. In addition, frequent communications and annual meetings of reference centre principal investigators will ensure study compliance. The centre's individual results of the study shall be owned by the centre. Each centre will provide copies of all results, including but not limited to case report forms, to the study coordination centre in Zurich. Owner of the overall data of the initiative is the EuroCYST Steering Committee.

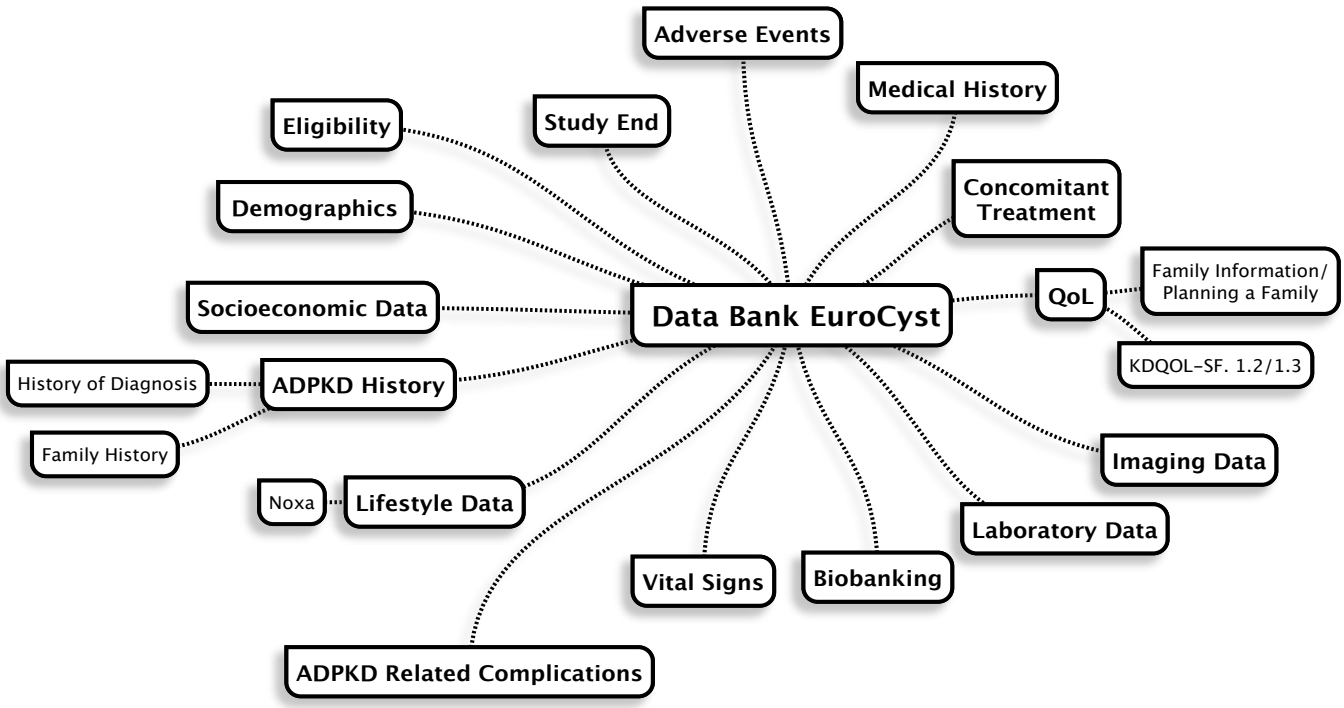

Figure 3: Databank

Statistical considerations

Formal sample size estimation has not been performed for this study. While chronic kidney disease cohorts currently aim to enrol at least 3,000 and up to 5,000 patients (CRIC, CKD JAC and GCKD) to identify valid associations in subgroups, the common genetic origin in APDKD allows important conclusions to be drawn using a smaller sample size.[149-151] On the other hand, groups of several hundred patients en- rolled in recent randomized controlled trials as well as already existing ADPKD cohorts have displayed large variability in the disease progression rate as assessed by the change in eGFR rate and TKV despite restrictive inclusion and exclusion criteria. Therefore, a larger sample size is required to account for the broader range in age and renal function in our cohort. A mixed-effects regression model will be used as the modelling framework, with a random effect for each patient (this allows correlation between repeated eGFR or TKV measurements in the same patient) and with fixed effects for time with a spline structure to model change in eGFR and TKV.

Study outcomes

The primary outcome measure of the study will be disease progression, assessed as change in eGFR (CKD-EPI) and change in TKV. Secondary outcome measure will be: first, onset and severity of ADPKD-related clinical outcomes, such as hypertension, albuminuria, renal urine concentrating ability, hematuria, renal pain, cyst infection and nephrolithiasis; secondly, self-reported health status, quality of life and pain; and thirdly, health-related resource use and ADPKD- related health burden.

Enrolment start

The study started enrolling patients in July 2013 and will run for 36 months.

Conclusion

The fragmentation of cohorts of ADPKD patients in Europe has been an obstacle to a better understanding of disease characteristics. Individual efforts in different countries often have little inter-changeability and it can be almost impossible to connect detailed clinical information held in one database with genetic information or biomaterial sample availability held in other databases. Our increasing knowledge of the basic biology of ADPKD has led to the identification of multiple novel targets in pre-clinical studies, which will need to be tested in patients. Positive results from recent clinical trials also now compel nephrologists to find new ways of risk stratification to identify patients at higher risk of disease progression and who may benefit most from early intervention. So far, limited data are available addressing patients' quality of life, disease-related health burden, health-care resource use and reproductive planning. Currently, available data regarding quality of life for ADPKD patients are limited and often only applicable to those on dialysis or transplanted patients.[22,152-154]

These issues motivated the EuroCYST initiative, which aims to build an ADPKD reference centre network in Europe in order to establish a large pan-European observational cohort that will serve as a scaffold and platform enabling researchers to study the pathogenesis, progression factors, mortality, co- morbidity as well as health economic issues relevant to ADPKD as a major cause of kidney disease. Although there is an interest of the pharmaceutical industry to establish ADPKD databases, an independent academic network with a transparent open access policy remains essential. The recent establishment of the ERA–EDTA Working Group on Inherited Kidney Disorders demonstrates the interest and need for a consolidated pan-European approach in the field of inherited kidney diseases at large.[155]

The establishment of such a cohort has the potential to strengthen European ADPKD investigators by harmonizing and standardizing research efforts and will guarantee that current and upcoming technologies to study chronic kidney disease can be applied to ADPKD patients across Europe. Affected patients and their relatives will benefit from the scientific-medical innovations by improved prevention and awareness, treatment of disease-specific complications and development of new diagnostic and therapeutic modalities.

ACKNOWLEDGEMENTS

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Urinary Biomarkers at Early ADPKD Disease Stage

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Katja Petzold participated in and contributed to the scientific conduction of the study and in the data analysis. The manuscript was written, the figures and tables were made by Katja Petzold.

Summary: ADPKD is a slowly progressing disease with a high inter- and intrafamilial disease course. The use of traditional markers like creatinine clearance and eGFR are inappropriate to assess disease state. We investigated urinary markers of tubular injury and their association with disease burden in ADPKD patients at early disease course. Our results indicate that the biomarkers urine osmolality, Urinary Albumin-to-Creatinine Ratio and urinary KIM-1 are independently associated with kidney size and should be further investigated to evaluate their property to assess disease state at early ADPKD stage.

Abstract

Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by a decline in renal function at late disease stage when the majority of functional renal parenchyma is replaced by cystic tissue. Thus, kidney function, assessed by estimated glomerular filtration rate (eGFR) does not well represent disease burden in early disease. Here, we investigated various urinary markers for tubular injury and their association with disease burden in ADPKD patients at early disease course.

Methods:

ADPKD patients between 18 and 40 years with an eGFR greater or equal to 70 ml per min per 1.73m^2 were eligible for this cross-sectional study. Urinary Neutrophil Gelatinase-Associated Lipocalin (NGAL), Kidney Injury Molecule-1 (KIM-1), and Uromodulin (UMOD) were investigated by Enzyme-Linked Immunosorbent Assay. Clara Cell Protein 16 (CC16) was investigated by Latex Immuno Assay. Cryoscopy was performed to assess urine osmolality and Urinary Albumin-to-Creatinine Ratio (UACR) was calculated. The association and the predictive properties of the markers on eGFR and height adjusted total kidney volume (htTKV) was evaluated using multiple regression analysis, incorporating different control variables for adjustment. Internal bootstrapping validated the obtained results.

Results:

In 139 ADPKD patients (age 31 ± 7 years, mean eGFR of 93 ± 19 ml per min per 1.73 m^2) the total kidney volume was negatively correlated with eGFR and UMOD and positive associated with age, UACR, KIM-1 and urine osmolality after adjustment for possible confounders. Urine osmolality and htTKV were also associated with eGFR, whereas no association of CC16, NGAL and UMOD with eGFR or htTKV was found.

Conclusion:

UACR and urinary KIM-1 are independently associated with kidney size but not with renal function in our study population. Urine osmolality was associated with eGFR and kidney volume following adjustment for multiple confounders. Despite statistical significance, the clinical value of our results is not yet conceivable. Further studies are needed to evaluate the property of the aforementioned biomarkers to assess disease state at early ADPKD stage.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common inherited kidney diseases. It is characterized by a decline of glomerular filtration rate at advanced disease stage and a high inter- and intrafamilial variability in age of end stage renal disease (ESRD) onset, implying a challenge to predict individuals' disease progression. The development and continued accretion of cysts, as the most prominent feature in ADPKD, leads to a massive enlargement of the kidney and, subsequently, to a loss of its function. So far, no disease modifying treatment is available, except the recent approval of the vasopressin V_2 receptor antagonist Tolvaptan in Japan. Due to glomerular hyperfiltration of the remaining nephrons kidney function stays stable over decades. Thus, traditional markers for kidney function like serum creatinine and estimated glomerular filtration rate (eGFR) have limited ability to accurately assess disease state and to predict progression in the early course of ADPKD. Increasing evidence suggests that total kidney volume qualifies as a marker for disease progression in ADPKD.[1] In fact, the disease state may be reflected more accurately by total kidney volume and kidney growth rate than renal functional parameters like eGFR or creatinine clearance.[89] Total kidney volume can be accurately assessed by Magnetic Resonance Imaging (MRI). However MRI derived kidney volume measurements are time and cost intensive, require high technical expertise and are not routine clinical practice.

Renal cystogenesis in ADPKD is a complex process, characterized by abnormalities in tubular cell proliferation, fluid secretion, extracellular matrix formation, and cell polarity.[156] The process results in an impaired filtration barrier, diminished tubular reabsorption, upregulation of tubular proteins and release of markers by recruited inflammatory cells, which can be detected in patients' urine.[157] Such markers should have the property to define the patients' state in a certain

disease condition, to predict prognosis, and/or to quantify the effect of a pharmacological approach. Mayeux et al defined type 0 biomarkers (diagnostic biomarkers) reflecting the natural history and correlating with clinical indices and biomarkers of type 1 (predictive biomarkers) capturing the effect of an intervention.[63,6] Biomarkers of type 0 that reflect tubular damage and have been under investigation in various settings of kidney disease are Neutrophil Gelatinase Associated Lipocalin (NGAL), Kidney Injury Molecule-1 (KIM-1), Uromodulin (UMOD), Clara Cell Protein 16 (CC16) and albuminuria.[158] NGAL has been extensively investigated as a biomarker, due to its rapid increase in different settings like acute kidney injury, cardiac surgery, and kidney transplantation.[113,159-162] KIM-1 does not occur in human urine under physiological conditions and has been described as progression marker in kidney disease.[89] UMOD, the most abundant protein in human urine, regulates tubular function and shows protective properties against uropathogenic Escherichia coli and nephrolithiasis.[115] Decreasing levels of urinary UMOD have been reported in various settings of chronic kidney disease (CKD), like glomerulonephritis, diabetic nephropathy or tubulointerstitial nephropathy.[115,118,163,164] Urinary CC16 is consistently associated with defective endocytic uptake by the proximal tubule. CC16 levels are increased in patients with diabetic and HIV-induced nephropathy, as well as in renal Fanconi syndrome.[128,165] There is an unmet need to discover new biomarkers that allow an easy and non-invasive assessment of ADPKD disease state. Here, we investigated the potential properties of the aforementioned markers for assessing disease state by evaluating their association with kidney volume and function in patients at early stage of ADPKD.

Methods

Study Subjects

Subjects of the well-described SUISSE ADPKD cohort were eligible for enrolment.[50,166] Male and female patients with proven ADPKD diagnosis, examined by kidney ultrasonography, according to Ravine criteria, and a positive family history were eligible when aged between 18 and 40 and presenting with an eGFR greater or equal to 70 ml per min per 1.73m^2 as shown in Table 1.[5] A proof of a mutation in the *PKD1* or *PKD2* genes was required for enrolment of patients without family history (sequencing analysis by Athena Diagnostics Inc., Worcester, MA, USA). The study was conducted according to the Declaration of Helsinki and Good Clinical Practice Guidelines and was approved by the local ethical board. All patients provided written informed consent.

Table 1: Eligibility criteria[19]

- Age 18 to 40
- GFR ≥ 70 ml per min per 1.73m^2 (Cockcroft-Gault formula)
  - Clinical diagnosis of ADPKD based on kidney imaging (modified Ravine criteria) and family history
  - Positive family history for ADPKD
    - patients < 30 years: ≥ 2 cysts in either kidney
    - patients ≥ 30 years: ≥ 2 cysts in each kidney
- Negative family history for ADPKD cystic kidney disease by sonography: proof of a mutation in the PKD1 or PKD2 gene
- Patient provided written informed consent

Study Procedure

Subjects were invited to the outpatient clinic at the Division of Nephrology (University Hospital Zurich). At study visit the medical history was obtained, including medication and ADPKD related complications. Blood pressure measurement was done in duplicate at each arm after 5 minutes of rest in sitting position using an oscillometric blood pressure device (Boso-Medicus, Jungingen, Germany). Hypertension was defined as systolic blood pressure above 140 mmHg and/or diastolic blood pressure above 90 mmHg or antihypertensive treatment. Fasting spot urine samples were collected after voiding the first urine of the day to measure creatinine beside the potential biomarkers. Blood samples were centrifuged and aliquoted, according to a standardized process, to obtain serum. Serum and spot urine aliquots were stored at –80°C before analysis.

Laboratory Analysis

At study visit, serum creatinine was measured according to modified Jaffé method traceable to an isotope-dilution mass spectroscopy reference. Estimated GFR was calculated by applying the CKD-EPI equation.[148] NGAL (BioPorto Diagnostics A/S, Hellerup, Denmark) and KIM-1 (R&D Systems Inc., Abingdon, UK) were analyzed using commercially available Enzyme-linked Immunosorbent Assays (ELISA) according to manufacturers protocol. UMOD was analyzed by a well established ELISA based on a sheep anti-human uromodulin antibody (K90071C; Meridian Life Science, Memphis, TN) as the capture antibody, a mouse monoclonal anti-human Tamm–Horsfall protein antibody (CL 1032A; Cedarlane Laboratories, Burlington, NC) as the primary antibody, and a goat anti-mouse IgG (H+L) horseradish peroxidase–conjugated protein (172.1011; Bio-Rad Laboratories, Inc., Hercules, CA) as a secondary antibody. Human uromodulin (AG 733, stock solution: 100 µg/ml; EMD Millipore, Temecula,

CA) was used to establish the standard curve.[167] CC16 was analyzed with continuous flow Latex Immuno Assay (LIA) and an assayable concentration of CC16 between 0.3 and 40 µg/L.[168] Albuminuria was assessed using Synchron Systems for Microalbumin (Beckman Coulter Inc., Brea, California, USA). The urinary albumin-to-creatinine ratio (UACR) was calculated as follows: Albumin (mg/dl) x 1/creatinine (mg/dl) x 1000 µg/mg. The analysis of urine osmolality was performed by cryoscopy using a freezing point depression Advanced® 2020-BIO Multi-Sample Osmometer (Advanced Instruments Inc., Norwood, Massachusetts, USA). All samples were handled in a uniform way and underwent no freeze-thaw cycle before analyzed in duplicate.

Magnetic Resonance Imaging

Patients underwent magnetic kidney imaging without contrast media according to a standardized imaging protocol. The imaging was performed using a Signa Excite HDx system (GE Healthcare, Waukesha, WI, USA) and signal perception was obtained with an eight-channel antero-posterior-phased array surface coil. Trans-axial sequences consisted of two breathhold T1-weighted fast-spoiled gradient echo sequences with 3 and 4 mm slice thicknesses. Additionally, a trans-axial T2-weighted fast spin echo sequence with respiratory triggering was performed with 3 mm slice thickness. Right and left kidney volumes were measured and calculated using the Advantage Windows workstation (4.4 GE Healthcare, Buc, France). Total kidney volume (TKV) was calculated by adding the volume of the left and right kidney. Measurements of renal volume were done in a blinded way by two trained and independent observers. The renal hilum and the vessels were excluded from renal volume calculation. Variability was calculated as concordance correlation coefficients (95% CI) and were 1.000 (0.999–1.000) for intraobserver and 0.996 (0.995 – 0.999) for interobserver correlations.[14]

Statistical Analysis

SAS 9.4 was used for data analysis. A plausibility check of the data preceded the statistical analysis. Univariate methods were used to characterize study population. Values are given in means with standard deviation. TKV and height adjusted kidney volume (htTKV) are reported as median because of a skewed distribution of these parameters. Median is reported with interquartile range.

Spearman's correlation coefficient (r) was calculated to describe the correlation between biomarkers and eGFR and htTKV. To calculate r, reflecting the relative variance part, all values of the parameters are sorted and given a rank.[169] A positive r indicates a concordant association, whereas a negative r stands for an opposing association.

The association of biomarkers with eGFR and htTKV was evaluated by incorporating different control variables for adjustment and following a multiple linear regression approach. A stratum specific correlation analysis was performed for binary and ordinal variables. Multiple regression analysis gives information about importance and size effect of the predictors on the response variable, and about the interaction between predictors. Prerequisites, like the independence of predictors to each other, were evaluated and fulfilled. The models were determined with scatterplots and correlation analysis to verify linearity between outcome variable and predictors. Subsequently the regression equation was formed. Adjusted R^2 and p-value were calculated as statistic measures. R^2, the coefficient of determination, reflects the proportion response variation that is explained by the predictors.[170] Predictor variables were selected according to logical considerations and added sequentially to the models. The models were compared using the Akaike information criterion (AIC). The AIC is based on the likelihood and determines which model is more likely to be correct and comes closer to the "truth".[171] The smaller the AIC value the more realistic the model is, assuming, that a robust model predicts the

data well containing preferably a low number of predictors meeting the requirement for parsimony and avoiding overfitting.[172] Bootstrapping was applied to estimate the 2.5th and 97.5th percentile confidence intervals for each model. Our dataset was used as a pool from which 500 new datasets of the same size were randomly drawn with replacement to internally validate our model results.

Results

Demographics

Between April 2006 and April 2011 139 ADPKD patients were consecutively enrolled in the study. The mean age was 31±7 years and 85 (61%) patients were male. Hypertension was present in 82 (78%) patients, and 80 (58%) patients received antihypertensive medication. Among those, 50 patients received angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs), 16 received diuretics and 14 were treated with calcium antagonists. The mean eGFR was 93±19 ml per min per 1.73 m^2 and 74 (53%) of the patients had an eGFR greater than 90 ml per min per 1.73 m^2. The median TKV was 860 cm^3 (IQR, 568 to 1191 cm^3) and 52 (37%) patients had a TKV greater than 1000 cm^3. The median htTKV was 455 cm^3 (IQR, 17 to 669 cm^3). The mean body mass index (BMI) was 24±4 kg per m^2, 5 (4%) patients had a BMI lower than 18.5 kg per m^2 and 53 (38%) patients a BMI above 25 kg per m^2 (Table 2).

Table 2: Characteristics of study cohort

	ADPKD **n = 139**
Age – years	31±7
Sex – no. (%)	
Female	54 (39)
Male	85 (61)
Body mass index – kg per m^2	24±4
BMI <18.5	5 (4)
BMI 18.5 – 25	81 (58)
BMI >25	53 (38)
eGFR – ml per min per 1.73m^2	93±19
CKD stage 1	74 (53)
CKD stage 2	61 (44)
CKD stage 3	4 (3)
TKV – cm^3	860 (568 to 1191)
htTKV – cm^3 per m	455 (317 to 669)
Hypertension – no. (%)*	
Yes	82 (78)
No	23 (22)
Blood pressure – mmHg	
Systolic	131±16
Diastolic	83±11
Antihypertensive Medication - no. (%)	
ACE / ARB	50 (36)
Calcium antagonist	14 (10)
Diuretics	16 (12)

Abbreviations: eGFR – estimated glomerular filtration rate. TKV – total kidney volume, htTKV – height adjusted total kidney volume, ACE – angiontensin converting enzyme, ARB – angiotensin II receptor blocker. Values are means ± standard deviation and numbers (percentage), TKV and htTKV are reported as median (interquartile range), *total number of observations = 105

Analysis of biomarker

The results of the urinary parameters osmolality, NGAL, KIM-1, UMOD, UACR and CC16 were tabulated for the complete cohort and stratified for eGFR and TKV (Table 3). Osmolality was measured in 139 spot urine samples. All other potential biomarkers were measured in 132 samples. The median urinary osmolality was 364 mosmol per kg H_2O (IQR, 257 to 533 mosmol per kg H_2O). The median values were 9.8 µg per g creatinine (IQR, 5.3 to 23.7 µg per g creatinine) for NGAL, 274.6 ng per g creatinine (IQR, 131.3 to 457.3 ng per g creatinine) for KIM-1, and 16.3 mg per g creatinine (IQR, 10.2 to 26.7 mg per g creatinine) for UMOD. In the whole cohort, UACR was 14.0 mg per g creatinine (IQR, 8.4 to 23.1 mg per g creatinine) and the median for CC16 was 2.8 µg per l per g creatinine (IQR, 2.0 to 6.2 µg per l per g creatinine). The median of KIM-1 was significantly higher among patients with TKV above 1000 cm^3 than among patients with TKV lower or equal 1000 cm^3. Osmolality, NGAL, UMOD, UACR and CC16 were similar among patients with an eGFR above 90 ml per min per 1.73m^2 and less or equal 90 ml per min per 1.73m^2.

Table 3: Biomarker Analysis

	n	Complete Cohort	eGFR >90 ml per min per 1.73m^2	eGFR ≤90 ml per min per 1.73m^2	TKV ≤1000 cm^3	TKV >1000 cm^3
Osmolality – mosmol per kg H_2O	139	364 (257 to 533)	377 (266 to 534)	360 (243 to 506)	330 (236 to 497)	417 (332 to 547)
NGAL – µg per $g_{creatinine}$	132	9.8 (5.3 to 23.7)	8.7 (4.7 to 22.8)	11.3 (5.8 to 24.3)	12.5 (5.3 to 24.3)	9.0 (5.3 to 21.3)
KIM-1 – ng per $g_{creatinine}$	132	274.6 (131.3 to 457.3)	272.0 (113.9 to 448.8)	274.6 (161.3 to 479.9)	211.6 (106.4 to 362.5)	391.3 (196.1 to 581.0)*
UMOD – mg per $g_{creatinine}$	132	16.3 (10.2 to 26.7)	18.1 (10.9 to 29.8)	14.8 (9.1 to 25.6)	19.4 (10.7 to 28.5)	13.6 (8.3 to 21.6)
UACR – mg per $g_{creatinine}$	132	14.0 (8.4 to 23.1)	11.6 (6.9 to 21.8)	15.4 (9.9 to 27.2)	12.0 (7.7 to 21.3)	15.2 (10.4 to 31.1)
CC16 – µg per l per $g_{creatinine}$	132	2.8 (2.0 to 6.2)	3.0 (2.0 to 5.9)	2.6 (2.0 to 6.2)	2.6 (2.0 to 4.8)	3.2 (1.7 to 8.9)

Abbreviations: NGAL – Neutrophil Gelatinase Associated Lipocalin, KIM-1 – Kidney Injury Molecule-1, UMOD – Uromodulin, UACR – Urinary Albumin-to-Creatinine Ratio, CC16 – Clara Cell Protein 16. Values are reported as median (interquartile range), * $p < 0.0$

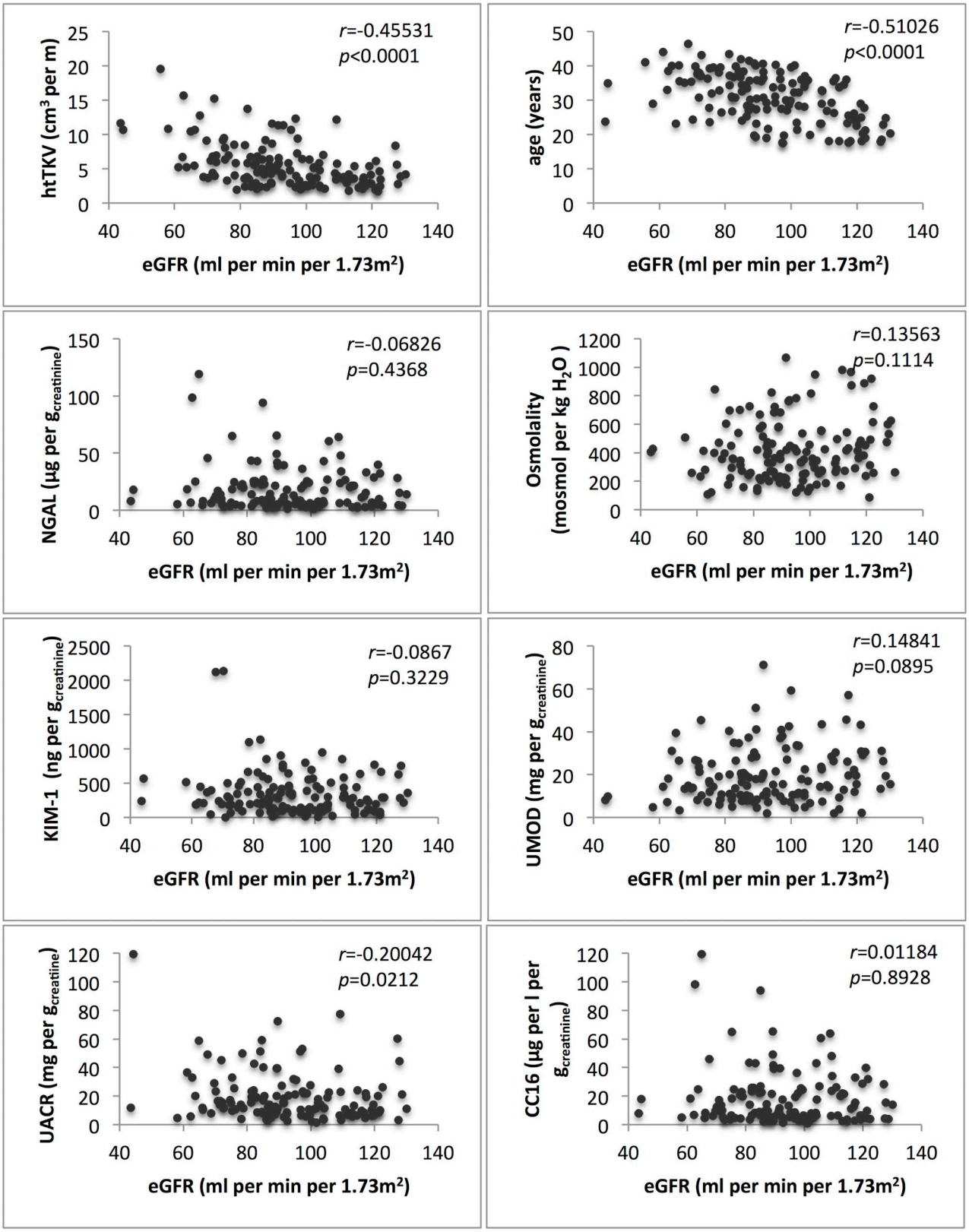
Correlation of biomarker with indices of disease progression

Table 4 shows the correlation of biomarkers with eGFR and TKV. Estimated GFR was negatively correlated with TKV ($r = -0.44508$, $p<0.05$), htTKV ($r = 0.45531$, $p<0.05$), age ($r = -0.51026$, $p<0.05$) and UACR ($r = -0.20042$, $p<0.05$). TKV was negatively correlated with eGFR ($r = -0.44508$, $p<0.05$) and UMOD ($r = -0.22771$ $p<0.05$) and positively correlated with age ($r = 0.22493$, $p<0.05$), urinary albumin ($r = 0.25524$, $p<0.05$), osmolality ($r = 0.1949$, $p<0.05$) and KIM-1 ($r = 0.32129$, $p<0.05$) (Table 4). Biomarker distribution is shown in figure 1 and 2.

Table 4: Spearman Correlation Coefficient *r*

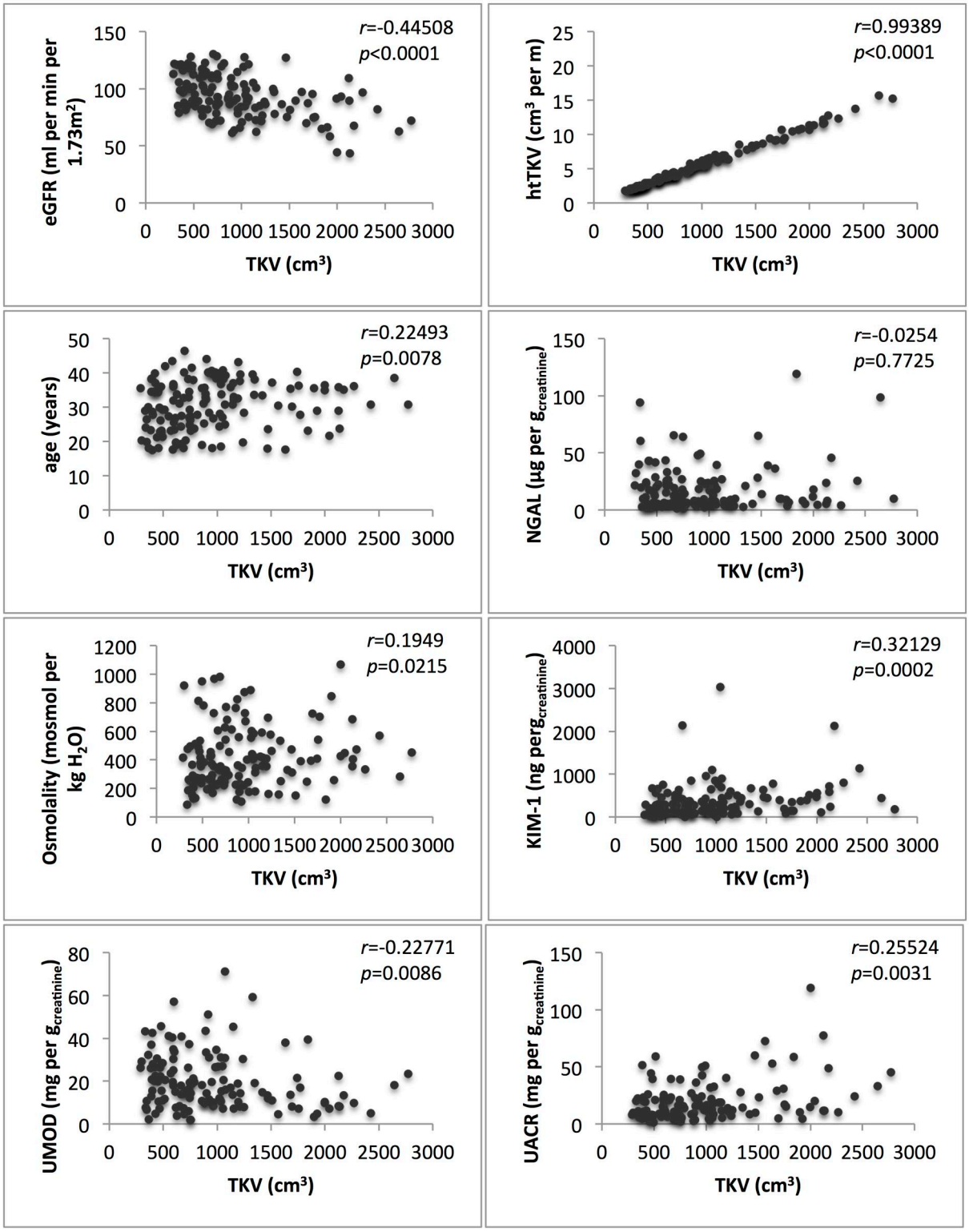
	eGFR	**TKV**	**htTKV**	**Age**	**NGAL**	**OSMOL**	**KIM-1**	**UMOD**	**UACR**	**CC16**
eGFR	1	-0.44508*	-0.45531*	-0.51026*	-0.06826	0.13563	-0.0867	0.14841	-0.20042*	0.01184
TKV	-0.44508*	1	0.99389*	0.22493*	-0.0254	0.1949*	0.32129*	-0.22771*	0.25524*	0.12924

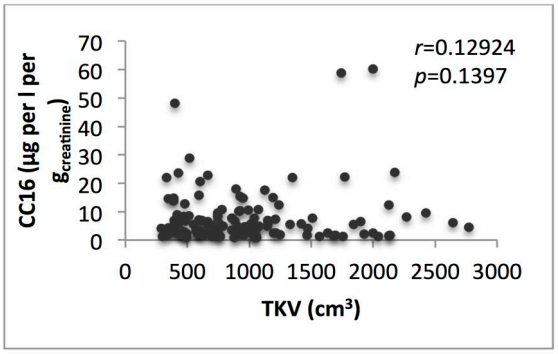
Abbreviations: eGFR – estimated glomerular filtration rate, TKV – total kidney volume, htTKV –height adjusted total kidney volume, NGAL – Neutrophil Gelatinase Associated Lipocalin, OSMOL – Osmolality, UMOD –Uromodulin, KIM-1 –Kidney Injury Molecule-1, UACR – Urinary Albumin-Creatinine-Ratio, CC16 – Clara Cell Protein 16. * $p < 0.05$



Abbreviations: htTKV – height adjusted total kidney volume, NGAL – Neutrophil Gelatinase Associated Lipocalin, KIM-1 – Kidney Injury Molecule-1, UMOD – Uromodulin, UACR – Urinary Albumin-to-Creatinine Ratio, CC16 – Clara Cell Protein 16

Figure 1: Estimated glomerular filtration rate (eGFR) and parameter distribution


r=-0.44508
p<0.0001
eGFR (ml per min per 1.73m²)
TKV (cm³)
r=0.99389
p<0.0001
htTKV (cm³ per m)
TKV (cm³)
r=0.22493
p=0.0078
age (years)
TKV (cm³)
r=-0.0254
p=0.7725
NGAL (µg per g creatinine)
TKV (cm³)
r=0.1949
p=0.0215
Osmolality (mosmol per kg H2O)
TKV (cm³)
r=0.32129
p=0.0002
KIM-1 (ng perg creatinine)
TKV (cm³)
r=-0.22771
p=0.0086
UMOD (mg per g creatinine)
TKV (cm³)
r=0.25524
p=0.0031
UACR (mg per g creatinine)
TKV (cm³)



Abbreviations: eGFR – estimated glomerular filtration rate, htTKV – height adjusted total kidney volume, NGAL – Neutrophil Gelatinase Associated Lipocalin, KIM-1 – Kidney Injury Molecule-1, UMOD – Uromodulin, UACR – Urinary Albumin-to-Creatinine Ratio, CC16 – Clara Cell Protein 16

Figure 2: Total kidney volume (TKV) and parameter distribution

Regression analysis for htTKV and eGFR as outcome parameter

Simple and multiple linear regression analysis were applied to delineate the independent associations of urinary biomarkers with eGFR and htTKV. Kidney volume is affected by a number of *a priori* known biological factors, e.g. age, gender, and glomerular filtration rate. Predictive variables were chosen in a predefined step-wise approach: In model 1 (Table 5) eGFR (β = -0.45968, p<.0001) was selected as a predictor; the term eGFR captures race, age and gender. The prognostic power of eGFR to predict htTKV is 20.6% (R^2 = 0.2055) with an AIC of -199.6. Bootstrapping revealed a percentile confidence interval of 0.0987 and 0.3374.

The selection of osmolality and UACR to model 1 as independent variables increased the R^2 to 0.3373 (percentile CI, 0.2306 – 0.4755), and the AIC to -210.7 (Table 6; Model 2). An increase of UACR (b = 0.20465, p<.0001) and urine osmolality (b = 0.32114, p<.0001) is independently of renal function, race, age, and gender associated with an increase in htTKV. All predictors of model 2 are independently predictors of htTKV at an alpha level of 0.1%. Estimated GFR has the most predictive power of all 3 variables in this model (β = -0.42435). The

standardized estimate β was calculated to evaluate the predictors independently of their transformation and their level of measurement.

Adjusted R^2 of model 3 was 0.3366 after selection of KIM-1, NGAL, UMOD, CC16 to eGFR, osmolality and UACR (Table 7). A percentile confidence interval of 0.2866 to 0.5278 was obtained in bootstrap validation. Out of the seven aforementioned variables, eGFR, osmolality, UACR and KIM-1 are major factors to predict htTKV. In model 3, the variable UACR is the second largest predictor variable with a β of 0.30403. Osmolality had a β of 0.21408, and KIM-1 a β of 0.18993. NGAL, UMOD and CC16 had low β-values and were minor determinants in the prognosis of htTKV. In model 3 osmolality, UACR and KIM-1 are positively correlated with kidney volume. The AIC of model 3 was -209.9. The additional selection of KIM-1, NGAL, UMOD and CC16 did not increase R^2 and did not change AIC.

Table 5: Simple linear regression height adjusted total kidney volume (transformed to log htTKV) Model 1

variable	**Parameter estimate b**	***p***	**Standardized estimate β**	**Standard Error**	**Adjusted R^2**	**AIC**
Intercept	2.20103	<.0001	0	0.1145	0.2055	-199.6
eGFR[1] – ml per min per 1.73m^2	-0.00007127	<.0001	-0.45968	0.00001181		

[1]square root transformed

Table 6: Multiple linear regression height adjusted total kidney volume (transformed to log htTKV) Model 2

variable	**Parameter estimate b**	***p***	**Standardized estimate β**	**Standard Error**	**Adjusted R^2**	**AIC**
Intercept	-0.29424	0.5704	0	0.51711	0.3373	-210.7
eGFR[1] – ml per min per 1.73m^2	-0.00006612	<.0001	-0.42435	0.00001149		
Osmolality[2] – mosmol per kg H_2O	0.32114	<.0001	0.30774	0.07825		
UACR[2] – mg per $g_{creatinine}$	0.20465	<.0001	0.31058	0.04985		

[1] square root transformed
[2] log transformed

Table 7: Multiple linear regression height adjusted total kidney volume (transformed to log htTKV) Model 3

variable	Parameter estimate b	*p*	Standardized estimate β	Standard Error	Adjusted R^2	AIC
Intercept	0.00847	0.9895	0	0.64145	0.3366	-209.9
eGFR[1] – ml per min per1.73m^2	-0.00006259	<.0001	-0.40173	0.00001166		
Osmolality[2] – mosmol per kg H_2O	0.2234	0.0186	0.21408	0.09366		
UACR[2] – mg per $g_{creatinine}$	0.20033	0.001	0.30403	0.05951		
KIM-1[2] – ng per $g_{creatinine}$	0.09432	0.0191	0.18993	0.0475		
NGAL[2] – µg per $g_{creatinine}$	-0.05253	0.2709	-0.09574	0.0397		
UMOD[2] – mg per $g_{creatinine}$	-0.04606	0.4653	-0.06085	0.06289		
CC16[2] – µg per l per $g_{creatinine}$	-0.00504	0.9212	-0.00794	0.05087		

[1] square root transformed
[2] log transformed

Subsequently different models were established to predict eGFR. In model 1 (Table 8) htTKV and osmolality were added on *priory* knowledge to predict eGFR. Both variables were independently associated with eGFR, and htTKV (β = -0.49803) had a larger association with eGFR compared with osmolality (β = 0.22936). The adjusted R^2 for this model is 0.2515 (percentile CI, 0.1588 – 0.3809) and thus approximately 25% of eGFR variation is explained by htTKV and osmolality.

In model 2 (Table 9) the predicting parameters htTKV, osmolality and UACR account for 22.09% of eGFR variation with an adjusted R^2 of 0.2209. A percentile confidence interval of 0.1319 to 0.3738 was obtained in bootstrap validation. The predictor htTKV has the largest impact on the outcome in this model (β = -0.48736) and osmolality showed the second largest value for standardized estimate (β = 0.21857). UACR has a comparably low β.

In model 3 (Table 10) the parameters htTKV, osmolality, UACR, NGAL, KIM-1, UMOD and CC16 were entered. Height adjusted total kidney volume (β = -0.47261; p<.0001) and osmolality (β = -0.27024; p = 0.006) were independently associated with changes of eGFR. The additional selection of NGAL, KIM-1, UMOD and CC16 resulted in a stable R^2 and AIC. Bootstrapping revealed a percentile confidence interval of 0.1674 to 0.4153.

Table 8: Multiple linear regression estimated glomerular filtration rate (square root transformed eGFR) Model 1

variable	Parameter estimate b	*p*	Standardized estimate β	Standard Error	Adjusted R^2	AIC
Intercept	4899.70827	0.1014	0	2970.99215	0.2515	2214.2
htTKV[1] – cm^3 per m	-3212.08387	<.0001	-0.49803	483.52856		
Osmolality[1] – mosmol per kg H_2O	1549.70316	0.0027	0.22936	506.54097		

[1] log transformed

Table 9: Multiple linear regression estimated glomerular filtration rate (transformed to square root eGFR) Model 2

Variable	Parameter estimate b	*p*	Standardized estimate β	Standard Error	Adjusted R^2	AIC
Intercept	4899.8604	0.1681	0	3534.68607	0.2209	2104.3
htTKV[1] – cm^3 per m	-3127.98641	<.0001	-0.48736	543.58333		
Osmolality[1] – mosmol per kg H_2O	1463.87536	0.0098	0.21857	557.90345		
UACR[1] – mg per $g_{creatinine}$	100.97551	0.7824	0.02388	364.80352		

[1] log transformed

Table 10: Multiple linear regression estimated glomerular filtration rate (transformed to square root eGFR) Model 3

Variable	**Parameter estimate b**	***p***	**Standardized estimate β**	**Standard Error**	**Adjusted R^2**	**AIC**
Intercept	170.01158	0.9697	0	4465.37589	0.2195	2108.4
htTKV[1] – cm^3 per m	-3333.33092	<.0001	-0.47261	564.97273		
Osmolality[1] – mosmol per kg H_2O	1809.95369	0.006	0.27024	646.61622		
UACR[1] – mg per $g_{creatinine}$	42.27981	0.9224	0.01	432.9461		
NGAL[1] – µg per $g_{creatinine}$	-136.7632	0.6812	-0.03884	332.06115		
KIM-1[1] – ng per $g_{creatinine}$	185.45311	0.5123	0.05818	282.16858		
UMOD[1] – mg per $g_{creatinine}$	798.52678	0.0675	0.16434	432.84451		
CC16[1] – µg per l per $g_{creatinine}$	-138.45145	0.6963	-0.03397	353.90411		

[1] log transformed

Discussion

The cystogenesis in ADPKD replaces functional renal parenchyma and leads to a loss of kidney function during patients' lifespan. Since GFR, a traditional parameter of renal function, is not able to accurately assess disease state in the early disease course, the interest in establishing urinary biomarkers for ADPKD has increased. In this cross-sectional study, we investigated the potential biomarkers osmolality, UACR, NGAL, UMOD, CC16, KIM-1 at a single time point in spot urine samples of 139 ADPKD patients with preserved renal function. Robust statistical approach demonstrated that urinary KIM-1, urinary osmolality and UACR are independently associated with kidney volume in our cohort of ADPKD patients.

An increase in urinary KIM-1, that is only fractionally expressed under physiological conditions, reflects tubular damage in the proximal S3 tubule segment as shown in acute and chronic kidney injury.[110] In our study, KIM-1 showed the strongest correlation with TKV. Multiple regression analysis revealed an independent correlation of KIM-1 with kidney volume, after adjustment for eGFR, osmolality, UACR, NGAL, UMOD, and CC16. In contrast, KIM-1 was not associated with renal function in multiple regression analysis adjusted for renal volume, osmolality, UACR, NGAL, KIM-1, UMOD, and CC16. KIM-1 expression was found in murine polycystic kidneys but not in wild type mice, driving the hypothesis that ADPKD patients may display higher urinary KIM-1 excretion.[110] KIM-1 has been identified as novel ciliary molecule. By interacting with the PKD2 Protein Transient Receptor Potential Polycystic 2, KIM-1 may be involved in cellular response to changes in extracellular fluid flow detected by the cilium.[109] To our knowledge urinary KIM-1 levels in ADPKD has only been reported once. In a study of Meijer et al increased KIM-1 levels in 24h urine samples of ADPKD patients were associated with total kidney volume, adjusted for age, gender and albuminuria compared with healthy volunteers.[89] In our study, KIM-1 was associated with TKV whereas renal function that is stable at early disease stage was not

associated with KIM-1. Given the limited available data of KIM-1 in APDKD patients, it is not possible to draw a conclusion about the property of KIM-1 as biomarker in early ADPKD.

A defect in osmoregulation has been shown in animal models of ADPKD as well as in patients.[173-175] The impaired capacity of urine concentration is an early manifestation and can be observed in children.[175,176] With our study we confirm the independent association of osmolality and TKV as shown by Ho et al.[175] Following multiple adjustments, urine osmolality is independently associated with kidney volume and function in our cohort. Hence, the assessment of spot urine osmolality may add further information for disease state assessment in ADPKD patients at early disease stage.

Albuminuria is known as marker for kidney damage for years. Urinary albumin excretion is routinely assessed in the diagnosis of renal injury, due its urinary appearance prior to GFR decline in different renal diseases. Albuminuria is associated with CKD progression, decreasing eGFR, increasing TKV, myocardial infarction and mortality.[14,25,81-84] In our study, UACR predicts the variation in htTKV but did not qualify as predictor for kidney function.

KIM-1, urine osmolality and UACR are independently associated with disease state in our study, but no association of NGAL, UMOD and CC16 with kidney volume and function at early ADPKD state was found. NGAL has been extensively studied as biomarker in acute kidney injury, but only limited data is available reporting NGAL levels in ADPKD.[113,159-162,177] Boligano et al reported markedly increased urinary NGAL levels in ADPKD patients at late disease state (eGFR 59 ± 38 ml per minute, Cockcroft-Gault formula) compared with healthy volunteers.[101] Parikh and colleagues investigated serum and urinary NGAL levels over a three year period in participants of the CRISP study, with kidney function comparable to our investigated cohort (creatinine clearance >70 mls/min). The majority of subjects showed normal to low baseline urinary NGAL levels. Even so the levels increased during the

study period with a drop at second and third year follow up, no association of NGAL quartiles with kidney volume and function were seen. Furthermore they showed highly elevated NGAL levels in cystic fluid in ADPKD patients compared to urine and serum values of healthy and of patients with AKI. The discrepancy of NGAL levels in urine and in cyst fluid may be attributable to a missing communication between tubules and detaching cysts in ADPKD.[177] In summary, NGAL levels may increase only in advanced disease and NGAL is not suitable to predict outcome at early stage when renal function is maintained.

To our knowledge, UMOD and CC16 levels have not been reported in ADPKD so far. In our study, no association of UMOD and CC16 with renal function and kidney volume was observed. Decreasing levels of urinary UMOD, which is the most abundant protein in human urine, have been reported in various settings of CKD.[115,118,163,164] Since the absolute values for urinary UMOD in our cohort are comparable with the ones reported in various cohorts, one could speculate that UMOD excretion decline starts in later stage of ADPKD.[126,178] CC16 is secreted by bronchial Clara cells and, after filtration, reabsorbed by receptor-mediated endocytosis in the early segments of the proximal tubule.[165] Hence, all disorders associated with defective proximal tubule endocytosis lead to the urinary loss of CC16.[179] The described lack of association of CC16 with kidney volume in contrast to KIM-1 probably reflects the functional segmentation of the proximal tubule, with endocytosis being particularly active in the S1-S2 segments whereas secretory pathways take place in the S3 segment.[180]

Our cross-sectional study has to be interpreted in the context of the study setting. We report independent association between biomarkers and outcome of a relatively high number of young ADPKD patients. We are not able to conclude about causal relationships between urinary biomarkers and outcome parameter. The parameters were only investigated at a single time point and we are not able to infer whether or not biomarker excretion precedes decrease in renal function and increase in kidney volume. Furthermore, our results are based on a

single centre in the absence of comparative groups of healthy volunteers or other CKD patients, making it impossible to assess the specificity of our findings for ADPKD. To partially account for the study limitations, we performed internal validation of our data set by bootstrapping. Despite adjustment for multiple confounders of our predictive models, we are not able to fully eliminate the potential for bias and confounding. We followed a robust and reliable statistical approach to investigate the diagnostic properties of different urinary biomarkers in a large cohort of ADPKD patients in early disease stage. Based on our results we hypothesize that osmolality, UACR and KIM-1 may have the property to assess disease state at early ADPKD disease course, whereas NGAL, UMOD and CC16 seem not to qualify as biomarkers. Further studies are necessary to define the biomarker properties of the investigated parameter to predict disease burden in ADPKD.

Disclosure

The authors declare no conflict of interest.

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Microhematuria in early ADPKD

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Katja Petzold designed and performed data analyis, wrote the manuscript and prepared all tables and figures.

Abstract

Background: Macrohematuria is common in Autosomal Dominant Polycystic Kidney Disease (ADPKD) and associated with renal volume as well as hypertension. In contrast, microhematuria is rarely investigated and commonly asymptomatic in ADPKD. In our study we investigate the prevalence and incidence of microhematuria, the determinants of microhematuria and potential association of microhematuria with outcomes of ADPKD.

Methods: 171 ADPKD patients with preserved kidney function underwent MRI assessment and physical examination at each study visit. Dipstick analysis and subsequent urine microscopy were performed to assess microhematuria in spot urine samples. Correlation and regression analyses were performed to identify disease indices that are associated with microhematuria in ADPKD.

Results: 171 ADPKD patients (31.8 ± 8.3 years, 59.1% male) with a mean eGFR of 92.6 ± 19.3 ml/min/1.73m^2, were followed over a median of 320 days. At study visits, a total of 50 events of microhematuria were recorded. Patients with microhematuria at baseline had lower eGFR, higher systolic blood pressure and higher values of proteinuria and albuminuria compared with patients without microhematuria at baseline. Microhematuria was, beside others, significantly negatively correlated with eGFR and positively correlated with macrohematuria and number of macrohematuric episodes. eGFR, systolic blood pressure and 24-hour albuminuria were significantly associated with microhematuria.

Conclusion: The prevalence and incidence of microhematuria in our cohort was low. Microhematuria was correlated with kidney function, systolic blood pressure, macrohematuria as well as albuminuria and proteinuria in our cohort of ADPKD patients. Importantly, increasing TKV, serum creatinine, protein-creatinine ratio as well as decreasing eGFR are predictive for microhematuria.

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent hereditary kidney disease, affecting approximately one in thousand life births.[66] The continuous development and progression of bilateral renal cysts destroys functional kidney parenchyma and leads to massive enlargement of the kidneys. Kidney function is stable over decades and starts to decline when cysts replace the majority of renal tissue, ultimately leading to kidney failure in 50% of the patients between their 50 and 60s years of life. Since ADPKD is a systemic disease, the clinical presentation is manifold. The increased mortality in ADPKD is attributed to cardiac abnormalities and intracranial aneurysm beside others. Early clinical symptoms include hypertension, microhematuria and flank pain.[66] In contrast, nephrolithiasis, cyst hemorrhage and macrohematuria are more pronounced in advanced state and correlate with kidney size.[20,70,71] Urine sediment abnormalities like hematuria are a risk factor for an accelerated disease progression in ADPKD.[13,69,73-76] Macrohematuria, the visible presence of blood in urine, occurs in 30 to 50% of ADPKD patients.[69,70] In ADPKD, episodes of macrohematuria are associated with increased renal volume and hypertension. Early and recurrent episodes are associated with more severe disease course and worse renal survival.[9,71,77] Causes for macrohematuria in ADPKD are the rupture of cyst lining vessels due to overactive vascular endothelial growth factor resulting in enhanced angiogenesis in the kidney.[71,10] Furthermore nephrolithiasis and urinary tract infections may explain lower and upper urinary tract hematuria.[71] Under physiological conditions erythrocytes are present only to a small extent in human urine. However, the presence of heme in the tubular system is per se a risk factor and may exert cytotoxicity and promote kidney function deterioration.[181] Macrohematuria is usually short lived but tends to be recurrent in ADPKD. Macrohematuric durations exceeding one week need to be screened for neoplasm.[71,182] Microscopic hematuria in ADPKD is of non-glomerular origin, remains often undetected, and rarely reported. The course is often asymptomatic.[78] Up to 60% of ADPKD patients undergo at least one episode

of micro- or macrohematuria.[77,13] The aim of the study was to characterize ADPKD patients in terms of microhematuria and to identify the role of microhematuria with the outcome of ADPKD patients.

Methods

Study subjects

Subjects included in this study belong to the well-described SUISSE ADPKD cohort, an ongoing observational longitudinal study conducted at the University Hospital of Zurich, Switzerland. [14,50] Since March 2006, patients diagnosed with ADPKD according to Ravine criteria, aged 18 to 40 years and with an eGFR of minimum 70 ml per min per 1.73m^2 (according to CKD-EPI formula) qualified for inclusion.[5] Patients were gradually enrolled in the study. The study was conducted according to the Declaration of Helsinki and Good Clinical Practice Guidelines. The local ethical board approved the study and all patients provided written informed consent.

Study procedure

Study subjects were invited to the outpatient clinic at the Division of Nephrology (University Hospital Zurich). At study visit the medical history was obtained, including medication and complications related to ADPKD. A brief physical examination including blood pressure measurement was performed. Blood pressure was measured using an oscillometric blood pressure device (Boso-Medicus, Jungingen, Germany) in duplicate at each arm after 5 minutes of rest in sitting position. Hypertension was defined as systolic blood pressure above 140 mmHg and/or diastolic blood pressure above 90 mmHg or antihypertensive treatment. Fresh morning midstream spot urine and blood samples were collected at study visit. Prior to the study visit all patients collected 24-hour urine.

Laboratory measurements

Serum creatinine and urinary creatinine were analyzed using the modified Jaffé method traceable to an isotope-dilution mass spectroscopy reference. Renal function was assessed using the CKD-EPI formula and CKD stages were defined according to KDIGO

2012 CKD Guideline for CKD stage 1 to 4.[16] Urinary protein was assessed using turbidimetry. Urinary albumin was analyzed with immunoturbidimetry. The analysis of urine osmolality was performed by cryoscopy.

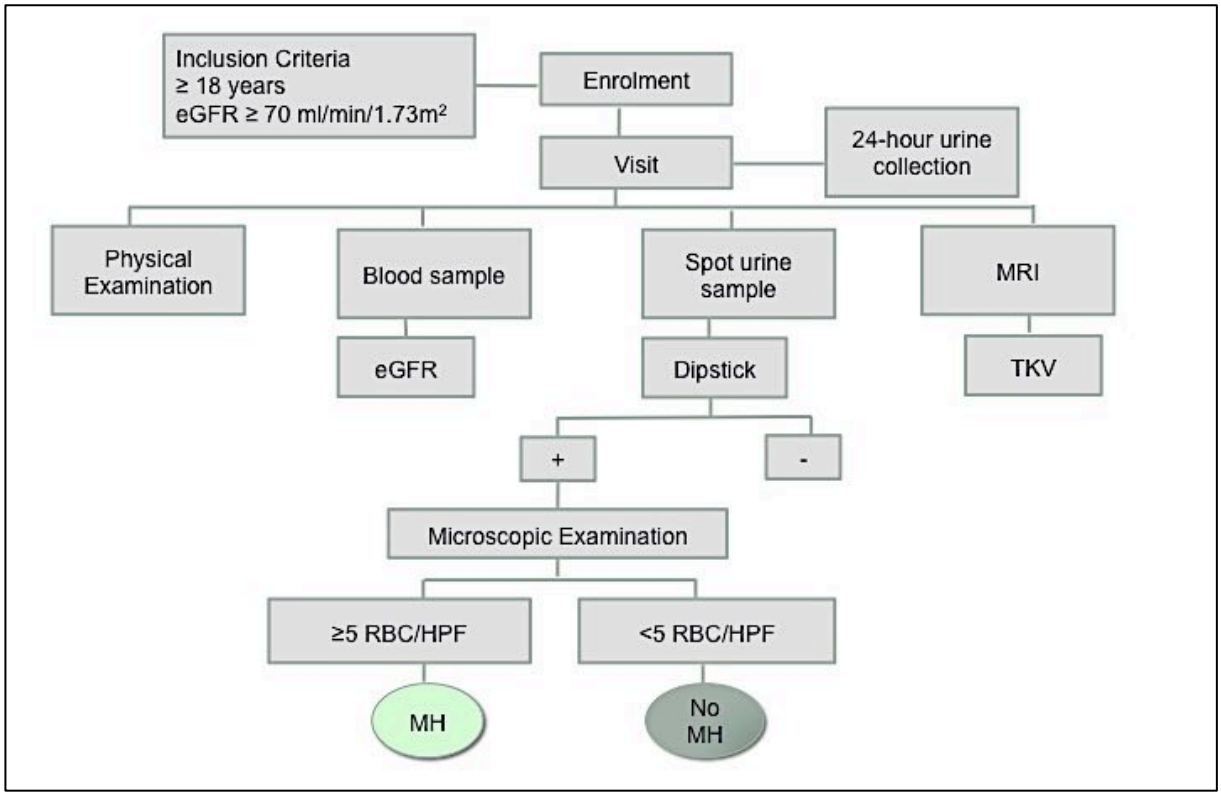
MRI and kidney volumetry

Patients underwent Magnetic Resonance Imaging in the absence of contrast media according to a standardized protocol. Two independent trained observers in a blinded fashion assessed kidney volume in stereological manner. Variability was calculated as concordance correlation coefficients (95% CI) and were 1.000 (0.999–1.000) for intraobserver and 0.996 (0.995 – 0.999) for interobserver correlations.[14] Total kidney volumes (TKV) were calculated by adding the volume of right and left kidney.

Urinalysis

Fresh morning midstream spot urine samples were processed according to European Urinalysis Guidelines.[183] The dipstick method (Combur 10 dipsticks, Roche Diagnostics, Mannheim, Germany) was applied to analyze all spot urine samples. Miditron Junior II (Roche Diagnostics, Mannheim, Germany), a semi-automated reflectance photometer was used to analyze the presence of erythrocytes, despite other parameters. The reference range for erythrocytes is 0 to 5 erythorcytes per µl (Ery/µl) with a practical detection limit of 5 Ery/µl or 0.03 mg/dl Hb (corresponds to 10 Ery/µl) according to the manufacturers protocol. A positive dipstick analysis required subsequent microscopic examination. 10 ml of urine samples positive for erythrocytes were centrifuged for 5 minutes at 1,500 revolutions per minute (rpm). Urine sediment was obtained by removing the supernatant and analyzed by a trained observer using a phase contrast microscope (Leica Microsystems, Wetzlar, Germany) at a magnification of x400 to measure erythrocytes, leukocytes, epithelial cells, crystals, casts, bacteria and yeast in number per high power field (HPF). Results derived from 10 evenly

distributed HPFs. Results ≥ 5 erythrocytes per HPF were classified positive for microhematuria, according to in-house standards. A sediment sample with a positive result was considered as one event of microhematuria.



Abbreviations: eGFR – estimated glomerular filtration rate, MRI – magnetic resonance imaging, TKV – total kidney volume, RBC/HPF – red blood cells per high power field, MH – Microhematuria

Figure 1: Flowchart of study process

Statistical Analysis

SPSS 21 and SAS 9.4 were used for data analysis and preparation of figures. The study population was characterized by using univariate methods. Results are given as means (± standard deviation) and median (interquartile range). Point-biserial correlation was applied to investigate correlation of various disease indices with microhematuria at baseline. Contingency tables were established to analyze the correlation of dichotomous variables. T-test for normal distributed parameter and Mann-Whitney-U-Test for not normal distributed variables have been applied to investigate differences in the stratified study population at baseline. Uni- and multivariate logistic regression analyses were used to investigate

determinants of microhematuria and the potential association with outcomes of ADPKD at baseline and at follow up. Univariate and multivariate logistic mixed effects models for correlated repeated measures data were done with the software package SAS 9.4. (SAS Institute Inc., Cary, NC) on a 64-bit workstation. The GLIMMIX procedure was used with maximum likelihood estimation with an adaptive Gauss-Hermite quadrature. Simplified models were selected over more complex models using the Akaike Information Criterion (AIC). P-values of <0.05 were considered significant.

Results

Demographics

A total number of 171 patients were included in the study. Data were collected from 626 visits. The mean age of the study population was 31.8 ± 8.3 years and 40.9% of the patients were female. The mean BMI of the study population at baseline was 24.3 ± 5.1 kg/m^2. With a mean eGFR of 92.6 ± 19.3 $ml/min/1.73m^{2,}$ the majority of the patients belonged to CKD stage 1 (eGFR $\geq$ 90 $ml/min/1.73m^2$) and 2 (eGFR = 60 – 89 $ml/min/1.73m^2$). 63.7% were classified as hypertensive, whereas 40.9% of the study population received antihypertensive treatment. The mean systolic and diastolic blood pressure was 135 ± 15 mmHg and 85 ± 10 mmHg, respectively. The median total kidney volume of the entire study population was 851.7 cm^3 (IQR, 544.4 – 1244.7). The 24-hour urine collection revealed a mean creatinine value of 7.12 ± 3.43 mmol/l. The mean urine osmolality in 24-hour urine collection was 446.0 (± 174.6) mosmol/kg H_2O. The median albumin-creatinine ratio was 9.20 mg/mmol (IQR, 5.40 – 22.05) in spot urine samples. Urine sediment analysis revealed calcium oxalate crystals in one patient and urate crystals in two patients at baseline visit. Questionnaires about ADPKD related symptoms and comorbidities showed that 23.9% of the study population experienced at least one event of macrohematuria during the course of ADPKD until baseline visit. Overall, these 26 patients reported 109

episodes of macrohematuria, with a maximum of 45 events per patient. Cyst infections have been reported by 25 (14.6%) patients and 62 (36.3%) experienced flank pain before baseline visit.

The study population was stratified according to the presence and non-presence of microhematuria at baseline visit, and into a group of patients without baseline microhematuria but with microhematuria during follow up. Patients without microhematuria were significantly different compared with patients with microhematuria at baseline in terms of serum creatinine and eGFR. Renal function, assessed as eGFR was significantly lower in patients with microhematuria compared with patients without microheamturia at baseline (81.6 ± 17.9 ml/min/1.73m^2 versus 93.6 ± 19.1 ml/min/1.73m^2). Furthermore, systolic blood pressure (145 ± 18 versus 134 ± 15 mmHg), 24-hour proteinuria (0.1063 ± 0.0753 versus 0.0661 ± 0.0355 g/l) and 24-hour albuminuria (34.85 (IQR, 10.78 – 101.15) versus 9.80 (IQR, 5.90 – 22.83) mg/l) were higher in patients with microhematuria compared with patients not presenting microhematuria at baseline visit. Patients that were positive for microhematuria during the follow-up are only significantly different for TKV (1137.4 (IQR, 868.6 – 1643.5) versus 791.1 (IQR, 520.1 – 1203.3) cm^3) compared to patients without microhematuria at baseline (Table 1).

The supplementary tables S1 and S2 show the stratification of the study population according to CKD stages and to TKV below and above median.

Table 1: Characteristics of patients with and without microhematuria at Baseline Visit and at Follow up

Characteristic	**Total** **N = 171**	Patients without microhematuria at BL N = 157	Patients with microhematuria at BL N =14	Patients with microhematuria at Follow up (but not at BL) N = 16
Age – years	31.8 ± 8.3	31.5 ± 8.3	34.8 ± 8.1	32.9 ± 6.2
Sex – no. (%)				
Female	70 (40.9)	67 (42.7)	3 (21.4)	5 (31.2)
Male	101 (59.1)	90 (57.3)	11 (78.6)	11 (68.8)
Weight – kg	76.5 ± 16.4	76.4 ± 16.5	77.9 ± 15.5	79.6 ± 24.9
BMI – kg/m^2	24.3 ± 5.1	24.2 ± 5.2	24.9 ± 4.3	25.1 ± 4.9
Serum creatinine – (µmol/l)	88.3 ± 20.2	87.1 ± 19.3	102 ± 25.9*	96.8 ± 28.6
eGFR – $ml/min/1.73m^2$	92.6 ± 19.3	93.6 ± 19.1	81.6 ± 17.9*	86.2 ± 17.9
CKD stage – no. (%)				
Stage 1	89 (52.0)	84 (53.5)	4 (28.6)	5 (31.2)
Stage 2	75 (43.9)	66 (42.0)	9 (64.3)	10 (62.5)
Stage 3	7 (4.1)	7 (4.6)	1 (7.1)	1 (6.3)
Blood pressure – mmHg				
Systolic	135 ± 15	134 ± 15	145 ± 18*	137 ± 15
Diastolic	85 ± 10	85 ± 10	88 ± 12	87 ± 9
Hypertension – no. (%)	109 (63.7)	97 (61.8)	12 (85.7)	11 (68.8)
Antihypertensive medication – no. of prescriptions (%)	70 (40.9)	59 (37.6)	11 (78.6)	5 (31.3)
ACE	66	56	10	4
Diuretics	22	17	5	1

others (BB, CCB, SIRA)	18	14	4	4
TKV – cm^3	851.7 (IQR, 544.4 – 1244.7)	791.1 (IQR, 520.1 – 1203.3)	1202.6 (IQR, 796.2 – 1202.6)	1137,4* (IQR, 868,6 – 1643,5)
24h Urine				
Creatinine – mmol/l	7.12 ± 3.43	7.12 ± 3.49	7.09 ± 2.48	7.56 ± 2.64
Protein – g/24h	0.142 ± 0.091	0.131 ± 0.070	0.253 ± 0.189*	0.120 ± 0.036
Albumin – mg/24h	22.45 (IQR, 13.20 – 54.05)	20.90 (IQR, 12.98 – 46.90)	83.25* (IQR, 23.53 – 158.25)	43.60 (IQR, 19.93 – 67.48)
Osmolality – mosmol/kg H_2O	446.0 ±174.6	446.5 ± 178.7	439.5 ± 116.6	467.6 ± 156.9
Spot urine				
Creatinine – mmol/l	11.56 ± 6.34	11.47 ± 6.34	12.53 ± 6.45	10.39 ± 5.82
Protein/Crea – g/mmol	0.009 (IQR, 0.006 – 0.012)	0.008 (IQR, 0.006 – 0.011)	0.016 (IQR, 0.007 – 0.021)	0.008 (IQR, 0.006 – 0.115)
Albumin/Crea – mg/mmol	2.000 (IQR, 0.900 – 4.200)	1.800 (IQR, 0.900 – 3.600)	6.850 (IQR, 2.425 – 12.250)	2.600 (IQR, 1.700 – 3.950)
Osmolality – mosmol/kg H_2O	562.78 ±204.77	561.48 ± 207.49	576.71 ± 178.71	511.9 ± 192.6
Urine sediment				
Calcium oxalate crystals –no. of patients	1	1	0	1
Urate crystals – no. of patients	2	0	2	0
Macrohematuria				
Present before/at BL – no.	26 (23.9)	20 (18.3)	6 (42.9)	5 (31.3)
Age at first event – years	26.3 ± 6.1	26.6 ± 6.2	25.1 ± 6.2	24.6 ± 7.2
Episodes until BL – no.	109	34	75	12

Cyst infection (present before/at BL) – no. of patients	25 (14.6)	20 (12.7)	5 (35.7)	5 (31.3)
Flank pain (present at or before/at BL) – no. of patients	62 (36.3)	53 (33.8)	9 (64.3)	8 (50.0)

Abbreviations: eGFR – estimated glomerular filtration rate. TKV – total kidney volume, ACE – angiotensin converting enzyme, BB=beta blocker, CCB=calcium channel blockers, BL – baseline visit. Values are means ± standard deviation and numbers (percentage), TKV, 24-hour albumin, protein-creatinine ratio, albumin-creatinine ratio are reported as median (interquartile range). *$p<0.05$

Prevalence and incidence of hematuria

Over the complete study duration, 50 events of microhematuria were recorded in 30 patients. The prevalence at baseline was 8.2% with 14 microhematuria events at baseline visit in 171 patients. The incidence rate for an episode of microhematuria was 29.2% in our cohort. The total study duration covered 108,675 patient days. The median follow up time was 320 days with a median visit interval of 190 days. 16 patients were positive for microhematuria during follow up without presenting microhematuria at baseline. Over the study duration, 7 patients experienced more than one event of microhematuria. The maximum number of events was 5, which was recorded in 2 patients. In total, 233 positive dipstick tests were detected over the study duration. Thereof, 50 (21.5%) were confirmed by microscopic evaluation of erythrocytes per HPF (Table 2). Due to the nature of the study with consecutive enrollment of patients, no patient was considered as lost of follow up during observation period.

Table 2: Positive dipstick vs. positive microscopic examination

visit	No of patients	Positive dipstick – no. (%)	Positive microscopic examination – no. (%)	Positive microscopic examination in relation to positive dipstick – %	Correlation between dipstick and microscopic examination
1	171	58 (33.9)	14 (8.2)	24.1	
2	149	55 (36.9)	9 (6.0)	16.4	
3	36	11 (30.6)	2 (5.6)	18.2	
4	52	26 (50.0)	6 (11.5)	23.1	
5	69	29 (42.0)	4 (5.8)	13.8	
6	52	22 (42.3)	5 (9.6)	22.7	
7	41	11(26.8)	4 (9.8)	36.4	
8	44	16 (36.4)	5 (11.4)	31.3	
9	12	5 (41.7)	1 (8.3)	20.0	
Σ		**233**	**50**	**21.5**	**r^2 = 0.383, p<0.05**

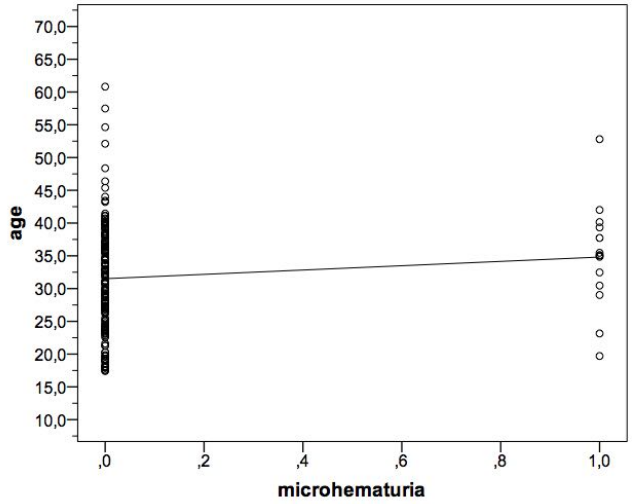
Correlation of microhematuria with disease indices of ADPKD at baseline

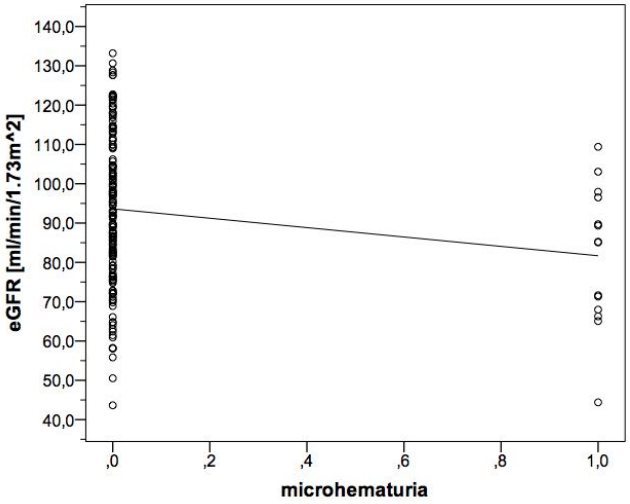
Point-biserial correlation and contingency tables were used to assess correlation of microhematuria with disease indices of ADPKD at baseline (Table 3, Figure 2). Thereof the number of episodes of macrohematuria showed highest correlation coefficient ($r^2 = 0.488$, $p = 0.011$) compared with other parameter. Furthermore renal function (eGFR) was significantly negatively correlated to microhematuria at baseline ($r^2 = - 0.171$, $p = 0.025$). Systolic blood pressure was significantly correlated with microhematuria ($r^2 = 0.183$, $p = 0.016$), whereas diastolic blood pressure was not. A positive correlation of 24-hour albuminuria ($r^2 = 0.337$, $p = 0.010$), and 24-hour proteinuria ($r^2 = 0.245$, $p = 0.002$) was found. The albumin-creatinine ratio from spot urine was also significantly correlated with microhematuria ($r^2 = 0.213$, $p = 0.007$). The establishment for contingency tables and appropriate bar graphs (Figure 3), depicting the correlation between two dichotomous parameters, manifested a positive correlation of high significance between macrohematuria and microhematuria ($r^2 = 0.193$, $p = 0.0045$).

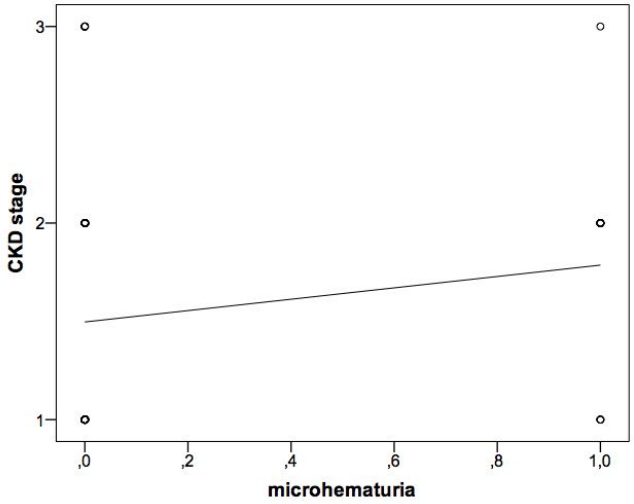
Table 3: Correlation of disease indices with microhematuria at baseline

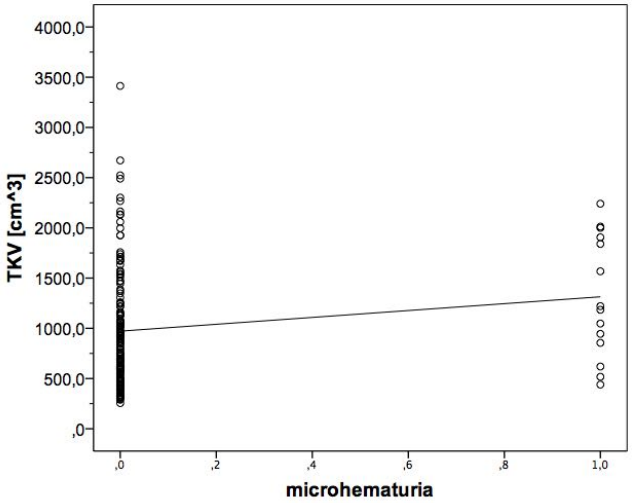
	age	eGFR	CKD stage	TKV	Hyper-tension	Bp sys	Bp dia	Macro-hematuria	Episodes of Macro-hematuria	24h Albuminuria	24h Proteinuria	Spot urine Alb/Crea	Spot urine Prot/Crea	Flank pain	Cyst infection
r^2	0.109	- 0.171	0.138	0.147	0.136	0.183	0.090	0.193	0.488	0.337	0.245	0.213	0,124	0.092	0.136
p	0.156	**0.025**	0.073	0.055	0.075	**0.016**	0.240	**0.045**	**0.011**	**0.010**	**0.002**	**0.007**	0.132	0.343	0.159

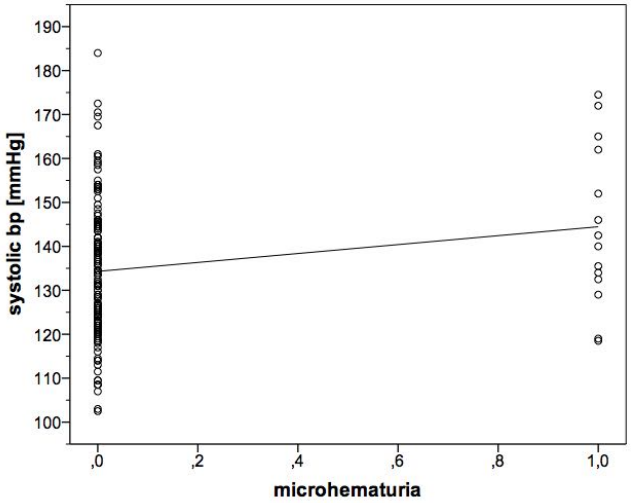
Abbreviations: eGFR—estimated glomerular filtration rate, TKV—total kidney volume, Bp sys—systolic blood pressure, Bp dia—diastolic blood pressure, Alb/Crea—albumin-creatinine-ratio, Prot/Crea—protein-creatinine-ratio.

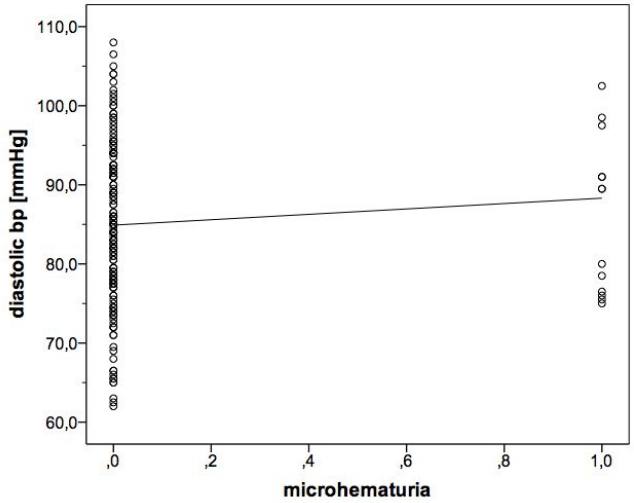

age
70,0
65,0
60,0
55,0
50,0
45,0
40,0
35,0
30,0
25,0
20,0
15,0
10,0
,0
,2
,4
,6
,8
1,0
microhematuria


eGFR [ml/min/1.73m^2]
140,0
130,0
120,0
110,0
100,0
90,0
80,0
70,0
60,0
50,0
40,0
,0
,2
,4
,6
,8
1,0
microhematuria


CKD stage
3
2
1
,0
,2
,4
,6
,8
1,0
microhematuria


TKV [cm^3]
4000,0
3500,0
3000,0
2500,0
2000,0
1500,0
1000,0
500,0
,0
,0
,2
,4
,6
,8
1,0
microhematuria


systolic bp [mmHg]
190
180
170
160
150
140
130
120
110
100
,0
,2
,4
,6
,8
1,0
microhematuria


diastolic bp [mmHg]
110,0
100,0
90,0
80,0
70,0
60,0
,0
,2
,4
,6
,8
1,0
microhematuria

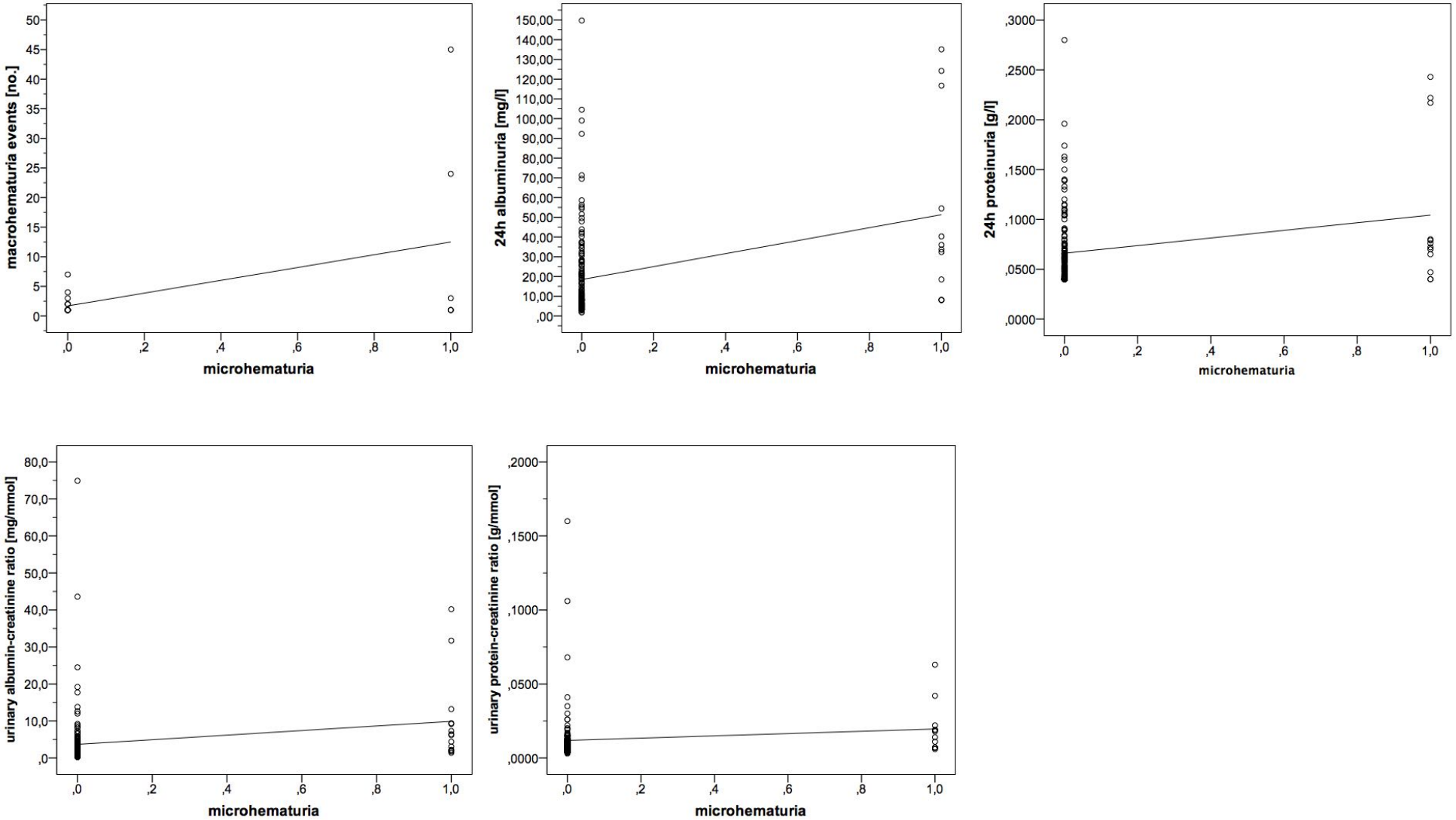


Figure 2: Correlation of microhematuria with disease indices of ADPKD

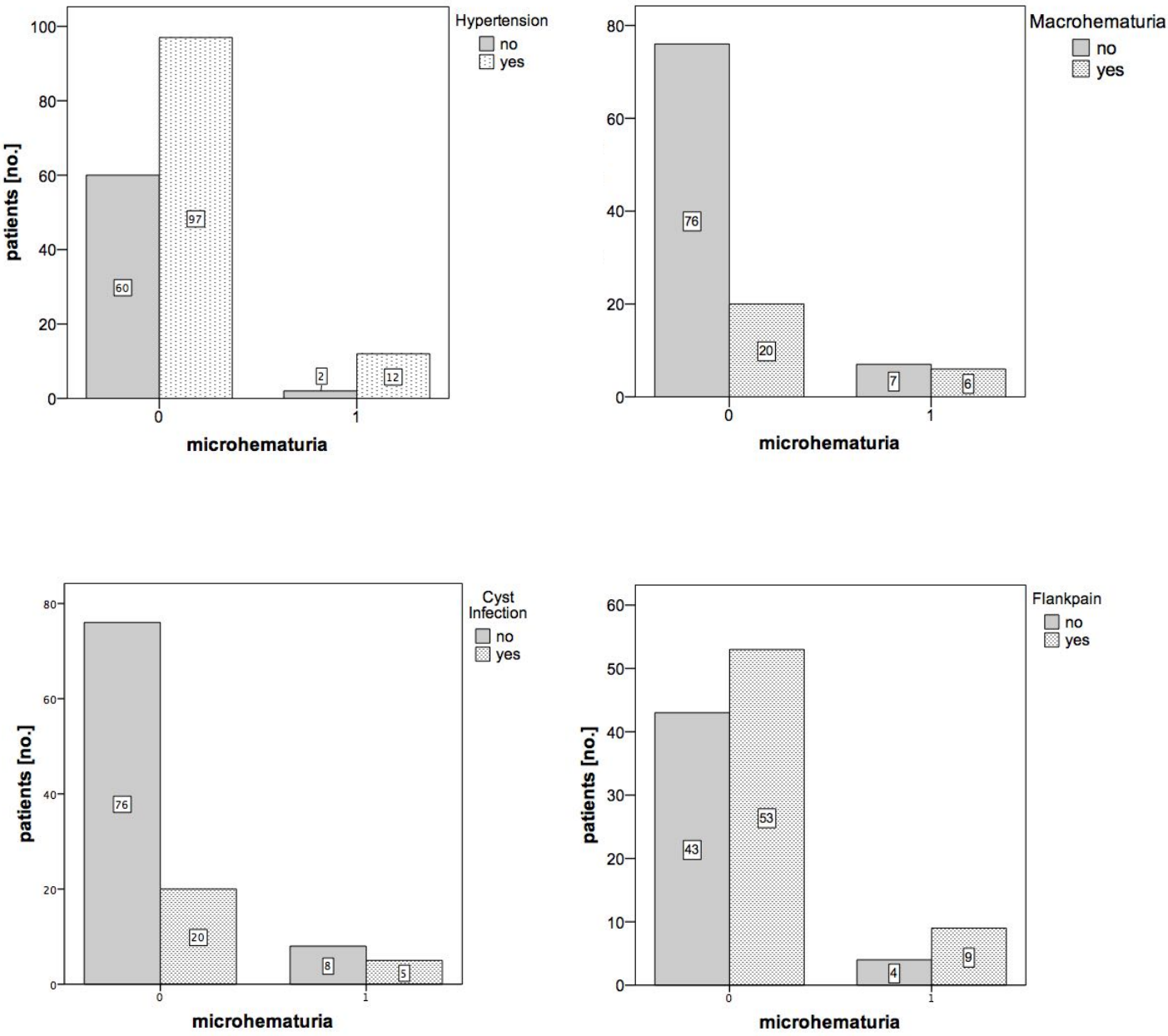


Figure 3: Bar graphs of binary disease indices in ADPKD

Uni- and multivariate Regression Analysis of parameters associated with microhematuria at baseline and follow up

Univariate and subsequent multivariate regression analysis was performed to identify the effect of various parameters on the occurrence of microhematuria. In the univariate analysis the continuous variables age, TKV, eGFR, blood pressure, 24h-albuminuria and the number of macrohematuric episodes were included as well as the dichotomous variable sex. Table 4 shows the results for univariate logistic regression with the regression factor B and the appropriate odds ratio. Renal function, expressed as eGFR (B = -0.034, p = 0.030), systolic blood pressure (B = 0.041, p = 0.020) and urinary 24-hour albumin excretion (B = 0.028, p = 0.001) show statistical significance in the univariate logistic regression. An increase in eGFR is associated with a decreasing risk to get microhematuria of about 3.3% (OR = 0.967) in our cohort. When systolic blood pressure increases by about 1 mmHg, the risk for microhematuria will increase by 4.2% (OR = 1.042). With each increase in 24-hour albuminuria, the risk for microhematuria will increase by 2.8% (OR = 1.028). Age, sex, TKV, diastolic blood pressure and macroscopic episodes are not significantly increasing the risk for experiencing an event of microhematuria in our cohort.

Table 4: Univariate logistic regression of various disease indices with microhematuria at baseline

Variable	**Regression coefficient B**	***p***	**Odds ratio (CI 95%)**
Age – age	0.045	0.158	1.046 (0.982 – 1.114)
Sex (male)	1.004	0.135	2.730 (0.733 – 10.169)
eGFR – ml/min/1.73m^2	- 0.034	**0.030**	0.967 (0.938 – 0.997)
TKV – cm^3	0.001	0.068	1.001 (1.000 – 1.001)
BP systolic – mmHg	0.041	**0.020**	1.042 (1.006 – 1.078)
BP diastolic – mmHg	0.032	0.241	1.033 (0.979 – 1.089)
Episodes of macrohematuria – no.	0.177	0.207	1.194 (0.907 – 1.572)
24-hour albuminuria – mg/24h	0.016	**0.001**	1.017 (1.007 – 1.027)
Spot urine Alb/Crea – mg/mmol	0.383	0.075	1.467 (0.963 – 2.237)

Subsequently multivariate logistic regression was performed (Table 5). In association model 1, including age, sex and TKV, age showed a significant influence to experience microhematuria (B = 0.034, p = 0.036), when adjusted for male and TKV. Interestingly, total kidney volume seems not to have any influence on the occurrence of microhematuria in univariate and multivariate analysis. In association 2, systolic blood pressure (B = 0.063, p = 0.013), remains significant following adjustment for eGFR, TKV and diastolic blood pressure. No significance was found in association 3, containing TKV, systolic and diastolic blood pressure, episodes of macrohematuria as well as 24h-albuminuria.

Table 5: Multivariate logistic regression of various disease indices with microhematuria at baseline

Variable	**Association 1**		**Association 2**		**Association 3**	
	Regression coefficient B	***p***	**Regression coefficient B**	***p***	**Regression coefficient B**	***p***
Age – years	0.034	**0.036**				
Sex (male)	0.853	0.070				
eGFR – ml/min/1.73m^2			- 0.032	0.076	- 1.385	0.998
TKV – cm^3	0.000	**0.000**	0.000	**0.039**		
BP systolic – mmHg			0.063	**0.013**	0.261	0.999
BP diastolic – mmHg			-0.050	0.209	- 4.534	0.996
Episodes of Macrohematuria – no.					5.556	0.996
Spot urine Alb/Crea – mg/mmol					7.402	0.996

Univariate logistic mixed effects models were established to evaluate the impact of disease indices on the outcome parameter microhematuria (Table 6). Longitudinally, eGFR is significantly inversly associated with microhematuria. An eGFR that is 5ml/min/1.73m^2 higher than the mean was associated with a decreased risk of microhematuria (OR = 0.792 (0.694 – 0.904), p = 0.0006). An increase in serum creatinine per 10 µmol/l was also associated with a higher risk for microhematuria (1.440 (1.181 – 1.756), p = 0.0003). The risk of microhematuria is increasing with spot urine albumin creatinine-ratio per 0.1 mg/mmol

increase (OR = 1.006 (1.001 – 1.010) $p = 0.0089$). Per each increase of kidney volume of 100 cm^3, the risk for experiencing microhematuria increased (1.088 (1.016 – 1.166), $p = 0.0154$).

Table 6: Longitudinal univariate logistic regression of various disease indices with microhematuria

Variable	Difference for odds ratio	Odds ratio (CI 95%)	*p*
Weight	10 kg	0.990 (0.847 – 1.658)	0.3214
Duration from baseline	365 days	1.044 (0.751 – 1.450)	0.9804
eGFR	5 ml/min/1.73m^2	0.792 (0.694 – 0.904)	**0.0006**
TKV	100 cm^3	1.088 (1.016 – 1.166)	**0.0154**
BP systolic	10 mmHg	1.222 (0.907 – 1.646)	0.1869
BP diastolic	10 mmHg	1.486 (0.946 –2.336)	0.0858
Serum creatinine	10 µmol/l	1.440 (1.181 – 1.756)	**0.0003**
Spot urine Alb/Crea	0.1 mg/mmol	1.006 (1.001 – 1.010)	**0.0089**

Subsequently, binary disease parameters were assessed for their association with microhematuria (Table 7). Thereof, flank pain (OR = 0.368 (0.186 – 0.730), $p = 0.0043$) and macrohematuria (OR = 0.299 (0.126 – 0.708), $p = 0.0043$) were significantly associated with microhematuria. Anorganic crystals, like calcium oxalate and urate crystals in patients' urine and cyst infection were not significantly associated with microhematuria.

Table 7: Longitudinal univariate logistic regression of binary disease indices with microhematuria

Variable	Difference for odds ratio	Odds Ratio (95%CI)	*p*
Urinary crystals	No urine crystals vs. urine crystals	0.184 (0.025 – 1.381)	0.0996
Cyst infection	No cyst infection vs. cysts infection	1.346 (0.290 – 8.657)	0.7542
Flank pain	No flank pain vs. flank pain	0.331 (0.148 – 0.738)	**0.0070**
Macrohematuria	No macrohematuria vs. macrohematuria	0.308 (0.108 – 0.880)	**0.0280**

The multivariate logistic regression based on longitudinal data, revealed that microhematuria is independently related to eGFR ($p = 0.0035$) and spot urine albumin to creatinine ratio ($p = 0.0352$) (Table 8). The addition of neither serum creatinine nor total kidney volume did

significantly improve the combined model given the strong correlation of serum creatinine and total kidney volume with eGFR (Table 9).

Table 8: Longitudinal multivariate logistic regression of eGFR and spot urine Alb/Crea with microhematuria

Variable	***p***	**AIC**
eGFR	0.0035	277.92
Spot urine Alb/Crea	0.0352	

Table 9: Longitudinal multivariate logistic regression of eGFR, TKV and spot urine Alb/Crea with microhematuria

Variable	***p***	**AIC**
eGFR	0.0319	275.19
TKV	0.5216	
Spot urine Alb/Crea	0.0318	

Discussion

Hematuria is a risk factor for disease progression in ADPKD. Whereas macrohematuria is known to occur in approximately 50% of patients and is associated with increased renal volume, only limited data is available on microhematuria in ADPKD. The data available indicates that microhematuria is associated with worse renal outcome, measured as increase in serum creatinine.[70]

In our study we investigated microhematuria in a cohort of young ADPKD patients with a mean eGFR of 92.6 ± 19.3 ml/min/1.73m^2. The effect of various parameters on the occurrence of microhematuria were investigated. In general, microhematuria may originate from any site of the urinary tract and may per se be a sign of an underlying disease. It is often detected accidently by urinary analysis. Regular screening is not performed routinely and microhematuria may occur without patients notice. Furthermore minor amounts of erythrocytes may be also excreted in healthy subjects. To prevent misclassification we performed a robust laboratory approach. A sediment analysis following each positive dipstick test was performed to distinguish between microhematuria and other reasons for erythrocytes in urine, like menstruation or urinary tract infections. False positive dipstick results were excluded from statistical analysis. We classified 5 or more erythrocytes per HPF as one event of microhematuria. However, different thresholds exist in the literature, ranging from 3 to 5 erythrocytes per HPF, challenging the comparison of results with other studies. We classified microhematuria as a dichotomous variable, with possible manifestation: yes or no.

The prevalence of microhematuria in the general population, as shown in population based studies, ranges from 0.19 to 16.1%.[79] Studies in older healthy males revealed a prevalence up to 21%, indicating a strong age dependency.[184] Our study population was followed over 108,675 patient days with a median follow up of 320 days. The prevalence of microhematuria at baseline of 8.1% might appear low. However, taken into account the characteristics of our population: patients are rather young and in general at an early stage of

their disease, with the majority of patients belonging to CKD stage 1 and 2. Nevertheless, even in this young study cohort we found significant differences after stratification in subgroups according to the appearance of microhematuria. Principally, due to the unnoticed appearance of microhematuria during disease course, investigators may probably miss events. Overall, we recorded 50 events of microhematuria. Compared to other studies indicating that up to 50% of ADPKD patients experience hematuria, often in recurrent episodes, the majority in our cohort experienced microhematuria at a single time point over the study duration. Seven patients exhibited 2 to a maximum of 5 events during the observation period. Only a small fraction of subjects belonged to CKD stage 3 at baseline (7 patients, 4,1%), as the most advanced stage in the present study at baseline visit.

In our cohort, microhematuria was significantly inversely correlated with eGFR. eGFR was also associated with microhematuria in baseline and longitudinal regression analysis. A decrease in renal function is significantly increased the risk of experiencing microhematuria in our cohort of young ADPKD patients. Therefore, we may conclude that beside macrohematuria also microhematuria as a potential risk marker for disease progression in addition to other well established risk factors.

Microhematuria was also correlated with macrohematuria and the number of macrohematuric events in our cohort. Also, the longitudinal regression analysis revealed an augmented risk for microhematuria. Based on these results it seems that macrohematuria may follow microhematura in a timely manner. However, patients without microhematuria at baseline reported 109 episodes of macrohematuria before baseline visit. Macrohematuria, due to cyst rupture or other events, may have detrimental effects on the kidney due to release of free iron and the occurrence of reactive oxygen species. Studies have shown that in patients the presence and number of events of macrohematuria are associated with hypertension and higher kidney volume.[34,70,79] In our study, systolic blood pressure was significantly correlated and associated with microhematuria at baseline. No association could be established in the

longitudinal analysis. Hypertension is a common and early sign of ADPKD in up to 80% of the patients with a mean age of 31 years.[10,34] The prevalence of hypertion in our cohort was 63.7%.

We found also a significant correlation between microhematuria and urinary parameters, like 24h-albumin excretion and albumin-creatinine and protein-creatinine ratio in spot urine. Under normal physiological conditions only 1 %, approximately 30 mg, of the filtered albumin is excreted per day.[85] Even the median of the complete cohort was in a normal range; 24-hour albuminuria was significantly higher in patients with microhematuria compared with patients without microhematuria. Patients with microhematuria at baseline visit can be classified as microalbuminuric, with a median albumin excretion above 45 mg per 24 hours.

To date, total kidney volume is a preferred parameter to accurately assess disease progression in ADPKD patients compared with eGFR. In our study, total kidney volume was not significantly correlated with microheamturia but was associated in longitudinal regression analysis. Each increase in kidney volume of about 100 cm^3 increased the risk of experiencing microhematuria by about 6.6%.

No data were available on the erythrocyte morphology to distinguish between glomerular or tubular occurrence of erythrocytes. Since macrohematuria is of glomerular origin in ADPKD, microhematuria is classified as tubular origin in the literature.[71,11,12] Since nephrolithiasis and urinary tract infections may explain lower and upper urinary tract hematuria in ADPKD, further studies are required to evaluate the exact cause of microhematuria in ADPKD patients.[71]

In summary, our study shows, that microhematuria is associated with different disease indices in a longitudinal fashion. Renal function, measured as eGFR, TKV as well as albumin-creatinine ratio were associated with microhematuria. Flank pain and macrohematuria episodes were predictive for microhematuria. Patients positive for

microhematuria may have an accelerated disease course in terms of eGFR, serum creatinine, 24-hour albuminuria and systolic blood pressure. If microhematuira would qualify as a marker to assess early disease state ADPKD has to be evaluated in further studies.

Acknowledgements

We thank Ruth Russi for technical support.

Conflict of interest

The authors declare no conflict of interest.

Supplement

Table S1: Baseline characteristics of the study population according to CKD stage

Characteristic	CKD stage 1 N = 89	CKD stage 2 N = 75	CKD stage 3 N = 7
Age – years	28.1 ± 6.7	35.0 ± 8.0	37.5 ± 9.2
Sex – no. (%)			
Female	39 (43.8)	29 (38.7)	2 (28.6)
Male	50 (56.2)	46 (61.3)	5 (71.4)
Weight – kg	73.3 ± 14.4	79.4 ± 17.7	87.3 ± 17.2
BMI – kg per m^2	23.4 ± 4.4	24.9 ± 5.6	27.5 ± 5.0
Serum creatinine – µmol/l	76.6 ± 12.4	97.6 ± 14.0	138.7 ± 29.5
eGFR – ml/min/1.73m^2	107.7 ± 11.2	78.5 ± 8.5	52.7 ± 6.5
Blood pressure – mmHg			
Systolic	134 ± 15	136 ± 15	131.8 ± 12
Diastolic	83 ± 11	87 ± 10	85.8 ± 7.1
Hypertension – no. (%)	49 (55.1)	53 (70.7)	7 (100)
Antihypertensive treatment – no. (%)	20 (22.5)	43 (57.3)	7 (100)
ACE – no. of prescriptions	18	41	7
Diuretics – no. of prescriptions	4	15	3
Others (BB, CCB) – no. of prescriptions	3	11	4
TKV – cm^3	648.9 (IQR, 468.9 – 944.9)	1035.8 (IQR, 649.5 – 1533.8)	1925.3 (IQR, 1251.9 – 2134.7)
24h Urine			
Creatinine – mmol/l	7.23 ±3.67	7.03 ± 3.29	6.67 ± 1.12
Protein – g/l	0.0669 ± 0.0400	0.0683 ± 0.0371	0.0979 ± 0.0718
Protein – g/24h	0.129 ± 0.072	0.146 ± 0.076	0.262 ± 0.269
Albumin – mg/l	8.90 (IQR, 5.25 – 8.90)	13.15 (IQR, 7.53 – 28.33)	32.70 (IQR, 4.40 – 54.40)
Albumin – mg/24h	18.40 (IQR, 11.60 – 46.20)	27.80 (IQR, 16.25 – 64.25)	42.30 (IQR, 12.98 – 178.88)
Osmolality – mosmol/kg H_2O	458.9 ± 191.2	433.2 ± 160.0	422.1 ± 92.7

Spot urine			
Creatinine – mmol/l	11.41 ± 6.85	12.02 ±5.89	8.56 ± 2.85
Protein/Crea – g/mmol	0.008 (IQR, 0.006 – 0.0110	0.009 (IQR, 0.006 – 0.012)	0.0180 (IQR, 0.0060 – 0.0353)
Albumin/Crea – mg/mmol	1.70 (IQR, 0.80 – 3.20)	2.20 (IQR, 1.00 – 4.85)	8.20 (IQR, 1.00 – 13.80)
Osmolality – mosmol/kg H_2O	576 .0 ± 231.2	559.5 ± 170.3	418.9 ± 119.2
Urine sediment			
Calciumoxalat crystals	0	1	0
Urate crystals	0	2	0
Microhematuria			
Present at BL visit – no. (%)	4 (4.5)	9 (12.0)	1 (14.3)
Macrohematuria			
Present before/at BL – no of patients (%)	9 (10.1)	14 (18.7)	3 (42.9)
Age at first event – years	26.3 ± 6.2	26.9 ± 6.1	23.8 ± 7.8
Episodes until BL – no.	10	69	26
Cyst infections (present before/at BL) – no. of patients	16	8 (10.7)	1 (14.3)
Flank pain (present before/at BL) – no. of patients	31	27 (36.0)	4 (57.1)

Abbreviations: eGFR – estimated glomerular filtration rate. TKV – total kidney volume, ACE – angiotensin converting enzyme, BB – beta blocker, CCB – calcium channel blockers, BL – baseline. Values are means ± standard deviation and numbers (percentage), TKV, 24-hour albumin, protein-creatinine ratio, albumin-creatinine ratio are reported as median (interquartile range).

Table S2: Baseline characteristics of the study population according to TKV.

	TKV below median (≤ 851.7 cm^3) N = 86	TKV above median (> 851.7 cm^3) N = 85
Age – years	29.8 ± 8.3	33.8 ± 7.9
Sex – no. (%)		
Female	47 (54.7)	23 (27.1)
Male	39 (45.3)	62 (72.9)
Weight – kg	71.4 ± 13.8	81.7 ± 17.2
BMI – kg per m^2	23.2 ± 4.8	25.4 ± 5.1
Serum creatinine – µmol/l	78.9 ± 14.5	97.8 ± 20.9
eGFR – ml/min/1.73m^2	100.9 ± 16.6	84.2 ± 18.2
CKD stage – no. (%)		
Stage 1	57 (66.3)	32 (37.3)
Stage 2	29 (33.7)	46 (54.1)
Stage 3	0 (0)	7 (8.2)
Blood pressure – mmHg		
Systolic	132 ± 16	139 ± 14
Diastolic	82 ± 9	89 ± 10
Hypertension – no. (%)	39 (45.3)	70 (82.4)
Antihypertensive treatment – no. (%)	21 (24.4)	49 (57.6)
ACE – no. of prescriptions	19	47
Diuretics – no. of prescriptions	6	19
Others (BB, CCB) – no. of prescriptions	3	15
24h Urine		
Creatinine – mmol/l	6.90 ± 3.81	7.34 ± 2.98
Protein – g/l	0.0626 ± 0.0367	0.0752 ± 0.0437
Protein – g/24h	0.118 ± 0.059	0.164 ± 0.109
Albumin – mg/l	8.00 (IQR, 5.10 – 16.30)	19.10 (IQR, 8.20 – 19.10)
Albumin – mg/24h	15.95 (IQR, 10.20 – 28.30)	34.05 (IQR, 17.45 – 76.00)
Osmolality – mosmol/kg H_2O	446.3 ± 195.0	445.8 ± 152.2
Spot urine		
Creatinine –mmol/l	12.40 ± 7.07	10.70 ± 5.42

Protein/Crea – g/mmol	0.008 (IQR, 0.005 – 0.0100)	0.010 (IQR, 0.007 – 0.013)
Albumin/Crea – mg/mmol	1.30 (IQR, 0.70 – 2.58)	2.90 (IQR, 1.50 – 5.50)
Osmolality – mosmol/kg H_2O	609.9 ± 225.3	515.1 ± 170.1
Urine sediment		
Calcium oxalate crystals	1	0
Urate crystals	0	2
Microhematuria		
Present before/at BL – no. of patients (%)	3 (3.5)	11 (12.9)
Macrohematuria		
Present before/at BL – no. of Patients (%)	8 (9.3)	18 (21.2)
Age at first event – years	27.5 ± 6.7	25.8 ± 6.1
Episodes until BL – no.	11	98
Cyst infections (present before/at BL) – no. of patients	13 (15.1)	12 (14.1)
Flank pain (present at or before/at BL) – no. of patients	20 (23.3)	42 (49.4)

Abbreviations: eGFR – estimated glomerular filtration rate. TKV – total kidney volume, ACE – angiotensin converting enzyme, BB – beta blocker, CCB – calcium channel blockers, BL – baseline. Values are means ± standard deviation and numbers (percentage), TKV, 24-hour albumin, protein-creatinine ratio, albumin-creatinine ratio are reported as median (interquartile range)

Publications, not contributing to this thesis

1. Katja Petzold, Andreas Serra, Aktuelle Therapiestudien für die Indikation zystischer Nierenerkrankungen, Der Nephrologe 09/2013; 2013(8):396-405. DOI: 10.1007/s11560-012-0733-9

2. Laura Rotar, Andreas Serra, Katja Petzold, Tolvaptan zur Therapie der autosomal-dominanten polyzystischen Nierenerkrankung - aktueller Stand, Schweiz Med Forum 04/2014; 2014(14 (16-17)):350-351

3. Daniela Spichtig, Hongbo Zhang, Nilufar Mohebbi, Ivana Pavik, Katja Petzold, Gerti Stange, Lanja Saleh, Ilka Edenhofer, Stephan Segerer, Jürg Biber, Philippe Jaeger, Andreas L Serra, Carsten A Wagner, Renal expression of FGF23 and peripheral resistance to elevated FGF23 in rodent models of polycystic kidney disease, Kidney International, 01/2014; DOI: 10.1038/ki.2013.526

4. Ivana Pavik, Philippe Jaeger, Lena Ebner, Carsten A Wagner, Katja Petzold, Daniela Spichtig, Diane Poster, Rudolf P Wüthrich, Stefan Russmann, Andreas L Serra, Secreted Klotho and FGF23 in chronic kidney disease Stage 1 to 5: a sequence suggested from a cross-sectional study. Nephrology Dialysis Transplantation, 11/2012; DOI: 10.1093/ndt/gfs460

5. Spithoven et al, on behalf of ERA-EDTA Registry and EuroCyst, Analysis of data from the ERA EDTA Registry indicates that conventional treatments for chronic kidney disease do not reduce the need for renal replacement therapy in Autosomal Dominant Polycystic Kidney Disease, Kidney Int. 2014 Dec;86(6):1244-52. doi: 10.1038/ki.2014.120. Epub 2014 May 14

6. Spithoven et al, on behalf of ERA-EDTA Registry and EuroCyst, Renal replacement therapy for ADPKD in Europe: prevalence and survival. An analysis of data from the ERA-EDTA Registry, Nephrol Dial Transplant. 2014 Sep;29 Suppl 4:iv15-25. doi: 10.1093/ndt/gfu0

Discussion and Conclusion

ADPKD is the most common inherited renal disease, affecting around 12,000 people in Switzerland. Half of the patients will turn to ESRD during disease course and will require renal replacement therapy. The generation and growth of bilateral cysts in the kidney start early in life, commonly in the absence of clinical signs. In absence of an established family history, ADPKD remains often undiscovered until disease related complications, like hypertension, pain, hematuria, urinary tract infections and others occur.

Research over the recent years has brought more insight into pathogenic processes underlying ADPKD and new drug targets were discovered. Despite the progress made, different features of ADPKD remain unexplained. The causes for a strong variability of disease course within the same family and the causes for interfamilial variability of disease course remain elusive.

Cystogenesis is continuously replacing functional renal parenchyma. The remaining functional nephrons tend to hyperfiltrate and thereby compensate for lost nephrons, explaining the apparently stable kidney function over decades. The traditional marker for kidney function, serum creatinine, will only change when cysts replace the majority of functional parenchyma. Also the eGFR, calculated from serum creatinine levels, stays within normal ranges and is not appropriate to assess the exact disease state. However, the assessment of disease state in early course is important: first, to define possible interventions, which are supposed to slow down progression and second, to identify patients that would benefit most from upcoming medical treatments.

Until very recently, no medication was approved for treatment of ADPKD in Europe. The application of the selective vasopressin 2 receptor antagonist Tolvaptan is now approved for its

ADPKD application in Japan, Canada and Europe. Tolvaptan reduced the growth rate in ADPKD and treatment was associated with lower disease burden in respect to pain compared with placebo group. Side effects, like polyuria and nocturia lead to higher discontinuation rates in the Tolvaptan group.

Other substances, targeting predominantly kidney volume, showed promising results in basic studies but failed to affect disease course under clinical evaluation.[48,50,51] The mTOR Inhibitors Sirolimus and Everolimus slowed cyst growth and preserved renal function in an animal model.[46,47,49] The failure in showing effects in clinical studies may be caused by dose limiting side effects, less follow up time and/or heterogeneity of study population in terms of baseline renal function and volume.[185]

The effect of synthetic long acting somatostatin analogues has been found by chance during treatment of endocrine tumors, where it reduced liver growth and decelerated kidney volume growth.[185,44] The results available so far are insufficient to finally evaluate the effect of somatostatin analogues on ADPKD progression. In summary, the treatment options for ADPKD are scarce and an unmet need exists to evaluate patients that will undergo treatment, once the options are available in clinics. Since early disease course and fast progressive patients will benefit most, reliable biomarkers have to be established to evaluate these patients.

Does conventional CKD treatment affect ADPKD outcome?

Standard care focuses mainly on the treatment of co-morbidities. Conventional treatment for CKD includes blood pressure control, RAAS inhibition, and low protein diet. These regimens seem not to be effective on the overall progression of ADPKD, since the incidence of ESRD in ADPKD did not substantially change in Europe between 1991 and 2010.[186] The incidence rates for patients starting RRT due to ADPKD in the US from 2001 to 2010 was 7.5 cases per million

per year. The age of ESRD onset was 55.7 ± 13.2 years in 2001 to 2002 and remained unchanged during the period of observation.[187] Results of the Danish National Registry on Regular Dialysis and Transplantation revealed an increase in RRT incidence from 6.45 cases per million in 1990 to 1995 and 7.59 million cases in 2002 to 2007. The mean age of onset of ESRD in Denmark increased by about 4.7 years to 60.6 years. The results of Orskov et al indicate an improved survival of patients before ESRD.[188]

A rigorous blood pressure control to 95 – 100/60 – 75 mmHg with ACE inhibitors may slow the growth in kidney volume compared with standard blood pressure targets (120 –130/70 – 80 mmHg).[17] The Kidney Disease: Improving Global Outcomes (KDIGO) Initiative is providing evidence based clinical guidelines for treatment of CKD patients since 1997 to improve the diagnosis and the treatment of chronic kidney disease.

The outstanding attribute of ADPKD compared with other types of chronic kidney disease is that the disease starts early in life. This can be seen as an advantage but also as a disadvantage. Discovered early, treatment for co-morbidities can take place and may delay renal function loss. Staying undiscovered, symptoms may only occur once renal parenchyma is destroyed and late treatment initiation will not improve outcome.

Overall, the management of co-morbidities in APDKD may delay the need of RRT but will not substantially change disease course nor halt disease progression, which is most important for patients displaying *PKD1* mutation, since they turn 20 years earlier to ESRD compared with *PKD2* mutation.

The need of prospective cohort studies

The research of ADPKD, especially in Europe, has been fragmented over the past years with several local smaller cohorts. Studies investigating disease progression were predominantly

retrospective and/or focussed on patients with advanced disease state. In contrast to the USA, in Europa no specific funding for research has been dedicated to ADPKD. Large prospective ADPKD studies, like CRISP and HALT-PKD in the USA are lacking in Europe. Large studies in Europe like the German CKD are not specifically investigating ADPKD.[151] Standardized common guidelines for the management of ADPKD patients are lacking. The EuroCYST initiative contributes to close this gap. The initiative will identify progression factors and biomarkers; assess disease stage specific mortality, morbidity and health costs. This knowledge should translate into new diagnostic and therapeutic modalities for patients with ADPKD. The inclusion of subjects in currently 10 countries will offer a cross section of the European ADPKD population that is followed in a standardized fashion. The large pan-European observational cohort will serve as a scaffold and platform enabling researchers to study the pathogenesis, progression factors, mortality, co-morbidity as well as health economic issues relevant to ADPKD as a major cause of kidney disease. The data set of the EuroCYST study has been carefully selected to comprise fields that, one the one hand have been investigated only novercally in the past, like socio-economic situation, QoL and noxa of ADPKD patients. On the other hand, it will be possible to harmonize our data with already existing databanks.

Primary outcomes of the study will be biomarkers of disease progression and the association thereof with the onset and severity of ADPKD related outcomes in a European cohort presenting an eGFR of minimum 30 ml per min per 1.73m^2 at baseline. To prevent selection bias and to represent early and advanced disease states an eGFR stratification at inclusion of 40 to 60% of enrolled subjects with eGFR above and under 60 ml per min per 1.73 m^2.

Beside the investigation of biomarkers in specimens of ADPKD patients a broad field of secondary objectives will be investigated in the EuroCYST study. The impact of disease on patients health status has seldom been investigated and insights of the QoL is predominantly

available from advanced stages and ESRD patients.[189,190] The HALT-PKD investigators hypothesized a diminished health related QoL (HRQoL) in advanced disease state, which is associated with TKV in CKD stage 1 to 4. In fact, ADPKD related symptoms were only weakly associated with HRQoL and HRQoL was not associated with TKV in this study.[22] ADPKD affects families and probably generations thereof. The communication of ADPKD within families may be difficult for patients and could lead to delay of discovery of ADPKD in siblings and probably delay medical treatment and intervention. We therefore conducted a questionnaire about planning a family and family information which will gain valuable information of the communication culture within families and the attitude towards ADPKD testing in respect to systematic imaging, genetic testing and the rate of incidental finding. The frequence of collecting blood and urine samples for biobanking and MRI should be intensified to have the possibility to accurately follow disease course and to investigate potentially biomarkers and their characterstic in ADPKD over a broad range of disease course. To even more strengthen the study, EuroCYST will follow the patients longitudinally and the duration and patient enrolment should be extended beyond the actual period of 3 annual follow up visits to follow the patients over a long disease duration and to specify co-morbidity and ADPKD related complications over the whole range of disease course.

Even so, the patient number of 1,100 is unique for a study on ADPKD in Europe, the quantity of enrolled subjects should be increased, by elevating the number of patients per study centre and/or by involvement of additional study centres in Europe. This will also give a broader picture of the patient characterstics throughout Europe.

Is TKV a prognostic marker?

Total kidney volume functions as surrogate endpoint for clinical studies in ADPKD. It is now commonly used by scientists but so far not accepted as primary endpoint in interventional studies by authorities like the FDA. The retardation in kidney volume growth and/or a total reduction in kidney volume are desired endpoints. Hard endpoints, like ESRD or death, are not practicable in ADPKD studies investigating early disease state. As shown in figure 3, renal function stays stable over decades and is therefore not an appropriate marker to assess the efficacy of an intervention.

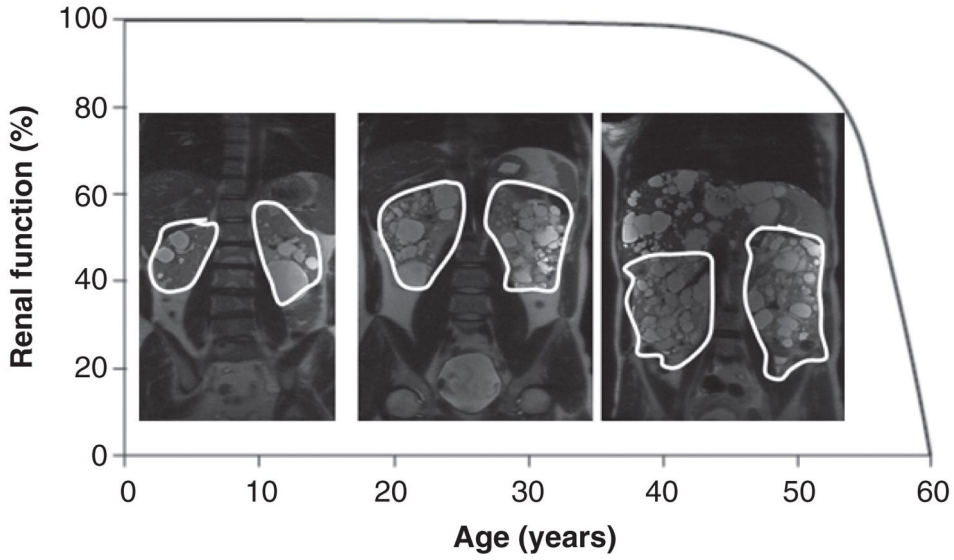


Figure 3: Renal function and TKV during disease course in ADPKD[36]

MRI and CT are desirable techniques to visualize and measure renal volume in ADPKD, whereas MRI has to be given preference due to lower radiation exposure. Patients with larger kidney volume at any given time point tend to be faster progressors with more rapid volume increase and more frequent occurrence of co-morbidities.[13] At the time-point new medical treatments will become available, the question has to be answered which patients to select for

treatment. Fast progressing patients in an early state of disease would probably benefit most from an interventional therapy. Therefore, it is crucial to accurately identify these patients. Volume visualizing in ADPKD is most accurate applying MR-technique, with detection of 2 mm small cysts.[14] Detrimental is its high technical demand, the cost and time intensity. Trained personnel has to perform the imaging in a standardized fashion applying defined settings, like sequences and slice thickness. Volume quantification is performed with stereological or midslice approaches with special programmes. Still, MR techniques are not clinical routine in all European countries. Irazabal et al developed a model to select patients for clinical studies based on kidney growth rate. Annual growth rates, starting from a theoretical baseline height adjusted renal volume of 150 ml, were categorized in slow, intermediate and fast volume progression. The annual growth rate in this study lay between of < 1.5% and > 6%. The established model predicts renal function based on annual percentage increase in kidney volume, calculated with an ellipsoid method. The rather new approach to quantify renal volume with the ellipsoid formula, using midslice tracing, is less time consuming compared with a stereological approach but probably less accurate.[138,140]

The annual average kidney growth is about 5.3% ± 4.0% and 5.36 ± 9.47%, assessed with MRI and stereological volume quantification.[13,14] There is a strong correlation between left and right kidney volume increase and between cyst and total kidney volume.[138] The increase in total kidney volume can be exclusively explained by cyst growth. Once total kidney volume reaches 1,500 cm^3, renal function decreases by about 5 ml eGFR per year.[13] Chapman et al predicted progression to CKD stage 3 within 8 years with baseline htTKV of 600 cm^3.[137] Also kidney length seems to predict development to stage 3 CKD.[139] Our results, described in section 2 confirmed the negative correlation of eGFR with TKV and htTKV, as it has been shown in several studies.[77,137,191] In fact, our statistical models seem to predict htTKV more precise than

eGFR, indicating htTKV being in general more accurate to predict diseases progression than eGFR does. A growing body of evidence shows that TKV is associated with ESRD prediction, hypertension, hematuria and proteinuria.[139]

Are urinary markers qualifying as prognostic biomarker in ADPKD?

The identification of biomarkers predicting renal function and kidney volume is described in the results section. We investigated parameters originating from the glomerulus, proximal and distal tubules that may qualify as biomarkesr for prognosis prediction in early ADPKD. There is an unmet need to establish biomarkers to assess disease severity and predict prognosis. We focused on urine spot samples that are obtainable in a non-invasive way and are a standard procedure in most nephrological practices. The potential and promising urinary biomarkers osmolality, NGAL, Umod, KIM-1, CC16 and UACR were tested to predict eGFR and kidney volume. These biomarkers may reflect renal glomerular and tubular damages (figure 4).

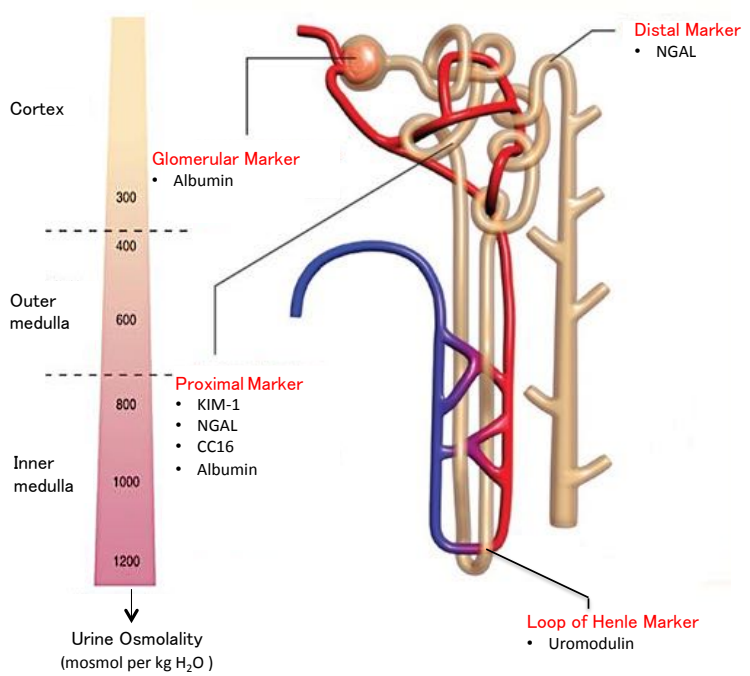


Figure 4: Potential urinary biomarkers in ADPKD

The mechanisms by which markers occur in urine are impairment of the filtration barrier; substances that are in healthy kidneys held back by the filtration barrier are now passing. Tubular reabsorption may be diminished or tubular proteins may be upregulated by injury and cell damage, like KIM-1. Furthermore recruited inflammatory cells can release markers. The complex process of cystogenesis in ADPKD is characterized by increased tubular cell proliferation, fluid secretion into cysts, and abnormal cell polarity.[192] Cysts develop from all parts of the nephron, and start to detach from the tubule by exceeding a diameter of 2 mm. Cysts ultimately leading to obstruction of tubules and urine flow through the compression of renal parenchyma. The damage leads to interstitial fibrosis and inflammation.[156]

In terms of biomarkers evaluation for ADPKD, we intended to combine several markers instead of using a single marker, assuming that modelling with more than one parameter will

allow more precise disease state and prognosis assessment. We investigated markers that are already well known to be abnormal in ADPKD, like urine osmolality and Albumin-Creatinine ratio with parameters that are so far un-investigated in ADPKD, like Umod and CC16. Urine osmolality in our study is, like shown by other investigators before, independently associated with kidney volume and kidney function.[175] Among our investigated urinary biomarkers, KIM-1 showed a strong correlation with TKV. NGAL has been extensively investigated as a biomarker, due to its rapid increase after kidney injury, in AKI, cardiac surgery and kidney transplantation.[113,159-162] Urinary NGAL levels are increasing and even more pronounced in fast progressing ADPKD compared to healthy but the evidence is per se sparse.[103] In our study NGAL was neither correlated with eGFR nor with TKV in univariante and multivariante regression analysis. The undetectable correlation may be caused by the properties of our cohort, composed of young patients with preserved renal function, as it was the case in the CRISP study. High levels of NGAL expression have been found in cystic epithelial cells in animal studies.[177] Decreasing levels of urinary Umod and positive correlation with renal function have been reported in various settings of CKD, like glomerulonephritis, diabetic nephropathy or tubulointerstitial nephropathy, potentially caused by loss of Umod secreting cells.[115,118,119,163,164] However, reporting Umod and CC16 levels in ADPKD for the first time, we are not able to detect associations with renal function or kidney volume.

In section three we investigated events of microhematuria in ADPKD in a longitudinal way. Microhematuria has been rarely investigated in ADPKD and requires time-consuming laboratory analyses to be detected compared with visible macrohematuria. Furthermore, micorhematuria can occur without patients notice. Episodes of gross hematuria before the age of 30 are associated with worse renal outcome compared to subjects without gross hematuric events.[9] The number of episodes and the age at which the first episode took place are predictors

for renal function decline.[34] With our study, we extend the spare data available about microhematuria in ADPKD, especially in early disease state and young patients. Microhematuria is associated with different disease indices at baseline and also in a longitudinal fashion. Renal function, as eGFR, TKV as well as albumin-creatinine ratio are associated with microhematuria over disease course. Also flank pain and macrohemautria episodes are predictive for microhematuria. We clearly found differences in patients with microhematuria compared with patients without microhematuria at baseline. Patients positive for microhematuria may have a accelerated disease course in terms of eGFR, serum creatinine, 24-hour albuminuria and systolic blood pressure. Screening for microhematuria is not a clinical routine. Also the cut-off values for erythrocytes per high power field for microhematuria classification differ between laboratories. If microhematuria qualifies as biomarker for ADPKD and should be investigated on a regular basis, it needs to be clearly defined in further studies.

Outlook

Preclinical and clinical research gained immense knowledge about the pathogenesis of ADPKD. Still, despite the progress made there is no cure for ADPKD. In this thesis, I described the setup and implementation of an international cohort of ADPKD patients. This study will provide further insights into the disease course and morbidities and co-morbidities in ADPKD. It will facilitate new techniques and the establishment of guidelines for ADPKD treatment. The deeper insight into patient disease burden will support to reach these goals. The EuroCYST initiative with 1,100 patients in 10 countries in Europe is an unique approach and will close the gap of fragmented research emerging from small and local cohorts in Europe.

The availability of upcoming interventional treatment presupposes the selection of the right patients. Fast progressing young patients will probably benefit most of an intervention halting PKD progression and sustaining renal function or at least substantially delay progression to ESRD. Developed models of biomarker will support the selection. As shown in this thesis, several markers seem to qualify as biomarker in ADPKD. The approach of establishing models containing a series of marker will precise the assertions of disease state and progression. The model should be precise as possible but should also be practicable in clinical routine. Further investigation is necessary to finally draw conclusions which markers add valuable information in evaluating patients' disease state and progression.

References

1. Torres VE, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. *Lancet*. 2007;369(9569):1287-1301. doi:10.1016/S0140-6736(07)60601-1.

2. Bastos AP, Onuchic LF. Molecular and cellular pathogenesis of autosomal dominant polycystic kidney disease. *Braz J Med Biol Res*. 2011;44(7):606-617.

3. Torres VE, Meijer E, Bae KT, et al. Rationale and design of the TEMPO (Tolvaptan Efficacy and Safety in Management of Autosomal Dominant Polycystic Kidney Disease and its Outcomes) 3-4 Study. *Am J Kidney Dis*. 2011;57(5):692-699. doi:10.1053/j.ajkd.2010.11.029.

4. Barua M, Pei Y. Diagnosis of autosomal-dominant polycystic kidney disease: an integrated approach. *Semin Nephrol*. 2010;30(4):356-365. doi:10.1016/j.semnephrol.2010.06.002.

5. Pei Y, Obaji J, Dupuis A, et al. Unified Criteria for Ultrasonographic Diagnosis of ADPKD. *Journal of the American Society of Nephrology*. 2009;20(1):205-212.

6. Pei Y. Practical genetics for autosomal dominant polycystic kidney disease. *Nephron Clin Pract*. 2011;118(1):c19-c30. doi:10.1159/000320887.

7. Bergmann C. ARPKD and early manifestations of ADPKD: the original polycystic kidney disease and phenocopies. *Pediatr Nephrol*. March 2014:1-16. doi:10.1007/s00467-013-2706-2.

8. Ong ACM, Harris PC. Molecular pathogenesis of ADPKD: the polycystin complex gets complex. *Kidney Int*. 2005;67(4):1234-1247. doi:10.1111/j.1523-1755.2005.00201.x.

9. Idrizi A, Barbullushi M, Petrela E, Kodra S, Koroshi A, Thereska N. The influence of renal manifestations to the progression of autosomal dominant polycystic kidney disease. *Hippokratia*. 2009;13(3):161-164.

10. Grantham JJ. Rationale for early treatment of polycystic kidney disease. *Pediatr Nephrol*. July 2014:1-10. doi:10.1007/s00467-014-2882-8.

11. Sweeney WE Jr, Avner ED. Diagnosis and management of childhood polycystic kidney disease. *Pediatr Nephrol*. 2011;26(5):675-692. doi:10.1007/s00467-010-1656-1.

12. Chang M-Y, Ong ACM. Autosomal dominant polycystic kidney disease: recent advances in pathogenesis and treatment. *Nephron Physiol*. 2008;108(1):p1-p7. doi:10.1159/000112495.

13. Grantham JJ, Torres VE, Chapman AB, et al. Volume progression in polycystic kidney disease. *N Engl J Med*. 2006;354(20):2122-2130. doi:10.1056/NEJMoa054341.

14. Kistler AD, Poster D, Krauer F, et al. Increases in kidney volume in autosomal dominant polycystic kidney disease can be detected within 6 months. *Kidney Int*. 2008;75(2):235-241. doi:10.1038/ki.2008.558.

15. Tahvanainen E, Tahvanainen P, Kääriäinen H, Höckerstedt K. Polycystic liver and kidney diseases. *Ann Med*. 2005;37(8):546-555. doi:10.1080/07853890500389181.

16. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward. *Kidney Int*. 2014;85(1):49-61. doi:10.1038/ki.2013.444.

17. Schrier RW, Abebe KZ, Perrone RD, et al. Blood pressure in early autosomal dominant polycystic kidney disease. *N Engl J Med*. 2014;371(24):2255-2266. doi:10.1056/NEJMoa1402685.

18. Levine E, Grantham JJ. Calcified renal stones and cyst calcifications in autosomal dominant polycystic kidney disease: clinical and CT study in 84 patients. *AJR Am J Roentgenol*. 1992;159(1):77-81. doi:10.2214/ajr.159.1.1609726.

19. Torres VE, Wilson DM, Hattery RR, Segura JW. Renal stone disease in autosomal dominant polycystic kidney disease. *Am J Kidney Dis*. 1993;22(4):513-519.

20. Nishiura JL, Neves RFCA, Eloi SRM, Cintra SMLF, Ajzen SA, Heilberg IP. Evaluation of nephrolithiasis in autosomal dominant polycystic kidney disease patients. *Clin J Am Soc Nephrol*. 2009;4(4):838-844. doi:10.2215/CJN.03100608.

21. Rahbari Oskoui F, Mittal A, Mittal P, Chapman A. Renal relevant radiology: radiologic imaging in autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol*. 2014;9(2):406-415. doi:10.2215/CJN.08940813.

22. Miskulin DC, Abebe KZ, Chapman AB, et al. Health-Related Quality of Life in Patients With Autosomal Dominant Polycystic Kidney Disease and CKD Stages 1-4: A Cross-sectional Study. *Am J Kidney Dis*. October 2013. doi:10.1053/j.ajkd.2013.08.017.

23. Cnossen WR, Drenth JPH. Polycystic liver disease: an overview of pathogenesis, clinical manifestations and management. *Orphanet J Rare Dis*. 2014;9(1):69. doi:10.1186/1750-1172-9-69.

24. Hogan MC, Abebe K, Torres VE, et al. Liver involvement in early autosomal-dominant polycystic kidney disease. *Clin Gastroenterol Hepatol*. 2015;13(1):155-64.e156. doi:10.1016/j.cgh.2014.07.051.

25. Torres VE, Chapman AB, Perrone RD, et al. Analysis of baseline parameters in the HALT polycystic kidney disease trials. *Kidney Int*. 2012;81(6):577-585. doi:10.1038/ki.2011.411.

26. Chapman AB, Johnson AM, Rainguet S, Hossack K, Gabow P, Schrier RW. Left ventricular hypertrophy in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 1997;8(8):1292-1297.

27. Faul C, Amaral AP, Oskouei B, et al. FGF23 induces left ventricular hypertrophy. *J Clin Invest*. 2011;121(11):4393-4408. doi:10.1172/JCI46122.

28. Chapman AB, Rubinstein D, Hughes R, et al. Intracranial aneurysms in autosomal dominant polycystic kidney disease. *N Engl J Med*. 1992;327(13):916-920. doi:10.1056/NEJM199209243271303.

29. Pirson Y. Extrarenal manifestations of autosomal dominant polycystic kidney disease. *Adv Chronic Kidney Dis*. 2010;17(2):173-180. doi:10.1053/j.ackd.2010.01.003.

30. Torra R, Sarquella J, Calabia J, et al. Prevalence of cysts in seminal tract and abnormal semen parameters in patients with autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol*. 2008;3(3):790-793. doi:10.2215/CJN.05311107.

31. Vora N, Perrone R, Bianchi DW. Reproductive issues for adults with autosomal dominant polycystic kidney disease. *Am J Kidney Dis*. 2008;51(2):307-318. doi:10.1053/j.ajkd.2007.09.010.

32. Kumar S, Adeva M, King BF, Kamath PS, Torres VE. Duodenal diverticulosis in autosomal dominant polycystic kidney disease. *Nephrol Dial Transplant*. 2006;21(12):3576-3578. doi:10.1093/ndt/gfl405.

33. Mekahli D, Bacchetta J. From bone abnormalities to mineral metabolism dysregulation in autosomal dominant polycystic kidney disease. *Pediatr Nephrol*. 2013;28(11):2089-2096. doi:10.1007/s00467-012-2384-5.

34. Schrier RW, Brosnahan G, Cadnapaphornchai MA, et al. Predictors of autosomal dominant polycystic kidney disease progression. *J Am Soc Nephrol*. 2014;25(11):2399-2418. doi:10.1681/ASN.2013111184.

35. Torres VE, Chapman AB, Devuyst O, et al. Tolvaptan in patients with autosomal dominant polycystic kidney disease. *N Engl J Med*. 2012;367(25):2407-2418. doi:10.1056/NEJMoa1205511.

36. Wüthrich RP, Mei C. Pharmacological management of polycystic kidney disease. *Expert Opin Pharmacother*. 2014;15(8):1085-1095. doi:10.1517/14656566.2014.903923.

37. Belibi FA, Wallace DP, Yamaguchi T, Christensen M, Reif G, Grantham JJ. The effect of caffeine on renal epithelial cells from patients with autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 2002;13(11):2723-2729.

38. Tanner GA, Tanner JA. Chronic caffeine consumption exacerbates hypertension in rats with polycystic kidney disease. *Am J Kidney Dis*. 2001;38(5):1089-1095. doi:10.1053/ajkd.2001.28614.

39. Gattone VH, Wang X, Harris PC, Torres VE. Inhibition of renal cystic disease development and progression by a vasopressin V2 receptor antagonist. *Nat Med*. 2003;9(10):1323-1326. doi:10.1038/nm935.

40. Hopp K, Hommerding CJ, Wang X, Ye H, Harris PC, Torres VE. Tolvaptan plus pasireotide shows enhanced efficacy in a PKD1 model. *J Am Soc Nephrol*. 2015;26(1):39-47. doi:10.1681/ASN.2013121312.

41. Torres VE, Wang X, Qian Q, Somlo S, Harris PC, Gattone VH. Effective treatment of an orthologous model of autosomal dominant polycystic kidney disease. *Nat Med*. 2004;10(4):363-364. doi:10.1038/nm1004.

42. Wang X, Gattone V, Harris PC, Torres VE. Effectiveness of vasopressin V2 receptor antagonists OPC-31260 and OPC-41061 on polycystic kidney disease development in the PCK rat. *J Am Soc Nephrol*. 2005;16(4):846-851. doi:10.1681/ASN.2004121090.

43. Meijer E, Gansevoort RT, de Jong PE, et al. Therapeutic potential of vasopressin V2 receptor antagonist in a mouse model for autosomal dominant polycystic kidney disease: optimal timing and dosing of the drug. *Nephrol Dial Transplant*. 2011;26(8):2445-2453. doi:10.1093/ndt/gfr069.

44. Serra AL, Petzold K. Aktuelle Therapiestudien für die Indikation zystischer Nierenerkrankungen. *Nephrologe*. 2013;8(5):396-405. doi:10.1007/s11560-012-0733-9.

45. Torres VE, Chapman AB, Devuyst O, et al. Tolvaptan in Patients with Autosomal Dominant Polycystic Kidney Disease. *N Engl J Med*. 2013;367(25):2407-2418. doi:doi: 10.1056/NEJMoa1205511.

46. Wahl PR, Serra AL, Le Hir M, Molle KD, Hall MN, Wüthrich RP. Inhibition of mTOR with sirolimus slows disease progression in Han:SPRD rats with autosomal dominant polycystic kidney disease (ADPKD). *Nephrol Dial Transplant*. 2006;21(3):598-604. doi:10.1093/ndt/gfi181.

47. Wu M, Arcaro A, Varga Z, et al. Pulse mTOR inhibitor treatment effectively controls cyst growth but leads to severe parenchymal and glomerular hypertrophy in rat polycystic kidney disease. *Am J Physiol Renal Physiol*. 2009;297(6):F1597-F1605. doi:10.1152/ajprenal.00430.2009.

48. Perico N, Antiga L, Caroli A, et al. Sirolimus therapy to halt the progression of ADPKD. *J Am Soc Nephrol*. 2010;21(6):1031-1040. doi:10.1681/ASN.2009121302.

49. Wu M, Wahl PR, Le Hir M, Wackerle-Men Y, Wüthrich RP, Serra AL. Everolimus retards cyst growth and preserves kidney function in a rodent model for polycystic kidney disease. *Kidney Blood Press Res*. 2007;30(4):253-259. doi:10.1159/000104818.

50. Serra AL, Poster D, Kistler AD, et al. Sirolimus and kidney growth in autosomal dominant polycystic kidney disease. *N Engl J Med*. 2010;363(9):820-829. doi:10.1056/NEJMoa0907419.

51. Walz G, Budde K, Mannaa M, et al. Everolimus in patients with autosomal dominant polycystic kidney disease. *N Engl J Med*. 2010;363(9):830-840. doi:10.1056/NEJMoa1003491.

52. Caroli A, Perico N, Perna A, et al. Effect of longacting somatostatin analogue on kidney and cyst growth in autosomal dominant polycystic kidney disease (ALADIN): a randomised, placebo-controlled, multicentre trial. *Lancet*. 2013;382(9903):1485-1495. doi:10.1016/S0140-6736(13)61407-5.

53. van Keimpema L, Nevens F, Vanslembrouck R, et al. Lanreotide reduces the volume of polycystic liver: a randomized, double-blind, placebo-controlled trial. *Gastroenterology*. 2009;137(5):1661–8.e1–2. doi:10.1053/j.gastro.2009.07.052.

54. Hogan MC, Masyuk TV, Page LJ, et al. Randomized clinical trial of long-acting somatostatin for autosomal dominant polycystic kidney and liver disease. *J Am Soc Nephrol*. 2010;21(6):1052-1061. doi:10.1681/ASN.2009121291.

55. Bukanov NO, Smith LA, Klinger KW, Ledbetter SR, Ibraghimov-Beskrovnaya O. Long-lasting arrest of murine polycystic kidney disease with CDK inhibitor roscovitine. *Nature*. 2006;444(7121):949-952. doi:10.1038/nature05348.

56. Albaqumi M, Srivastava S, Li Z, et al. KCa3.1 potassium channels are critical for cAMP-dependent chloride secretion and cyst growth in autosomal-dominant polycystic kidney disease. *Kidney Int*. 2008;74(6):740-749. doi:10.1038/ki.2008.246.

57. Sun Y, Zhou H, Yang B-X. Drug discovery for polycystic kidney disease. *Acta Pharmacol Sin*. 2011;32(6):805-816. doi:10.1038/aps.2011.29.

58. Katja Katja, Gansevoort RT, Ong ACM, et al. Building a network of ADPKD reference centres across Europe: the EuroCYST initiative. *Nephrol Dial Transplant*. 2014;29 Suppl 4(suppl 4):iv26-iv32. doi:10.1093/ndt/gfu091.

59. Strimbu K, Tavel JA. What are biomarkers? *Curr Opin HIV AIDS*. 2010;5(6):463-466. doi:10.1097/COH.0b013e32833ed177.

60. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69(3):89-95. doi:10.1067/mcp.2001.113989.

61. Puntmann VO. How-to guide on biomarkers: biomarker definitions, validation and applications with examples from cardiovascular disease. *Postgrad Med J*. 2009;85(1008):538-545. doi:10.1136/pgmj.2008.073759.

62. Waldmeier PC, Tatton WG. Interrupting apoptosis in neurodegenerative disease: potential for effective therapy? *Drug Discov Today*. 2004;9(5):210-218. doi:10.1016/S1359-6446(03)03000-9.

63. Mayeux R. Biomarkers: potential uses and limitations. *NeuroRx*. 2004;1(2):182-188. doi:10.1602/neurorx.1.2.182.

64. Shlipak MG, Day EC. Biomarkers for incident CKD: a new framework for interpreting the literature. *Nat Rev Nephrol*. 2013;9(8):478-483. doi:10.1038/nrneph.2013.108.

65. Reid CN, Stevenson M, Abogunrin F, et al. Standardization of diagnostic biomarker concentrations in urine: the hematuria caveat. *PLoS ONE*. 2012;7(12):e53354. doi:10.1371/journal.pone.0053354.

66. Takiar V, Caplan MJ. Polycystic kidney disease: pathogenesis and potential therapies. *Biochim Biophys Acta*. 2011;1812(10):1337-1343. doi:10.1016/j.bbadis.2010.11.014.

67. Dell KM. The spectrum of polycystic kidney disease in children. *Adv Chronic Kidney Dis*. 2011;18(5):339-347. doi:10.1053/j.ackd.2011.05.001.

68. Moreno JA, Martín-Cleary C, Gutiérrez E, et al. Haematuria: the forgotten CKD factor? *Nephrol Dial Transplant*. 2012;27(1):28-34. doi:10.1093/ndt/gfr749.

69. Milutinovic J, Fialkow PJ, Agodoa LY, Phillips LA, Rudd TG, Bryant JI. Autosomal dominant polycystic kidney disease: symptoms and clinical findings. *Q J Med*. 1984;53(212):511-522.

70. Gabow PA, Duley I, Johnson AM. Clinical profiles of gross hematuria in autosomal dominant polycystic kidney disease. *Am J Kidney Dis*. 1992;20(2):140-143.

71. Hogan MC, Norby SM. Evaluation and management of pain in autosomal dominant polycystic kidney disease. *Adv Chronic Kidney Dis*. 2010;17(3):e1-e16. doi:10.1053/j.ackd.2010.01.005.

72. Martinez JR, Grantham JJ. Polycystic kidney disease: etiology, pathogenesis, and treatment. *Dis Mon*. 1995;41(11):693-765.

73. Carville S, Wonderling D, Stevens P, Guideline Development Group. Early identification and management of chronic kidney disease in adults: summary of updated NICE guidance. *BMJ*. 2014;349(jul24 2):g4507-g4507. doi:10.1136/bmj.g4507.

74. Gabow PA, Johnson AM, Kaehny WD, et al. Factors affecting the progression of renal disease in autosomal-dominant polycystic kidney disease. *Kidney Int*. 1992;41(5):1311-1319.

75. Gabow PA. Autosomal dominant polycystic kidney disease. *N Engl J Med*. 1993;329(5):332-342. doi:10.1056/NEJM199307293290508.

76. Johnson AM, Gabow PA. Identification of patients with autosomal dominant polycystic kidney disease at highest risk for end-stage renal disease. *J Am Soc Nephrol*. 1997;8(10):1560-1567.

77. Masoumi A, Elhassan E, Schrier RW. Interpretation of renal volume in autosomal dominant polycystic kidney disease and relevant clinical implications. *Iran J Kidney Dis*. 2011;5(1):1-8.

78. Cohen RA, Brown RS. Clinical practice. Microscopic hematuria. *N Engl J Med*. 2003;348(23):2330-2338. doi:10.1056/NEJMcp012694.

79. Woolhandler S, Pels RJ, Bor DH, Himmelstein DU, Lawrence RS. Dipstick urinalysis screening of asymptomatic adults for urinary tract disorders. I. Hematuria and proteinuria. *JAMA*. 1989;262(9):1214-1219.

80. Vivante A, Afek A, Frenkel-Nir Y, et al. Persistent asymptomatic isolated microscopic hematuria in Israeli adolescents and young adults and risk for end-stage renal disease. *JAMA*. 2011;306(7):729-736. doi:10.1001/jama.2011.1141.

81. Tesch GH. Review: Serum and urine biomarkers of kidney disease: A pathophysiological perspective. *Nephrology (Carlton)*. 2010;15(6):609-616. doi:10.1111/j.1440-1797.2010.01361.x.

82. Fassett RG, Venuthurupalli SK, Gobe GC, Coombes JS, Cooper MA, Hoy WE. Biomarkers in chronic kidney disease: a review. *Kidney Int*. 2011;80(8):806-821. doi:10.1038/ki.2011.198.

83. Roscioni SS, de Zeeuw D, Hellemons ME, et al. A urinary peptide biomarker set predicts worsening of albuminuria in type 2 diabetes mellitus. *Diabetologia*. 2013;56(2):259-267. doi:10.1007/s00125-012-2755-2.

84. Chapman AB, Johnson AM, Gabow PA, Schrier RW. Overt proteinuria and microalbuminuria in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol*. 1994;5(6):1349-1354.

85. Gekle M. Renal Proximal Tubular Albumin Reabsorption: Daily Prevention of Albuminuria. *News Physiol Sci*. 1998;13:5-11.

86. Dickson LE, Wagner MC, Sandoval RM, Molitoris BA. The proximal tubule and albuminuria: really! *J Am Soc Nephrol*. 2014;25(3):443-453. doi:10.1681/ASN.2013090950.

87. Arroyo V. Human serum albumin: not just a plasma volume expander. *Hepatology*. 2009;50(2):355-357. doi:10.1002/hep.23006.

88. Nakamura T, Sato E, Fujiwara N, et al. Changes in urinary albumin excretion, inflammatory and oxidative stress markers in ADPKD patients with hypertension. *Am J Med Sci*. 2012;343(1):46-51. doi:10.1097/MAJ.0b013e31821f0552.

89. Meijer E, Boertien WE, Nauta FL, et al. Association of urinary biomarkers with disease severity in patients with autosomal dominant polycystic kidney disease: a cross-sectional analysis. *Am J Kidney Dis*. 2010;56(5):883-895. doi:10.1053/j.ajkd.2010.06.023.

90. Selistre L, de Souza V, Ranchin B, Hadj-Aissa A, Cochat P, Dubourg L. Early renal abnormalities in children with postnatally diagnosed autosomal dominant polycystic kidney disease. *Pediatr Nephrol*. 2012;27(9):1589-1593. doi:10.1007/s00467-012-2192-y.

91. Rodicio JL, Campo C, Ruilope LM. Microalbuminuria in essential hypertension. *Kidney Int Suppl*. 1998;68:S51-S54.

92. Levey AS, de Jong PE, Coresh J, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. In: Vol 80. 2011:17-28. doi:10.1038/ki.2010.483.

93. Peters HPE, Waanders F, Meijer E, et al. High urinary excretion of kidney injury molecule-1 is an independent predictor of end-stage renal disease in patients with IgA nephropathy. *Nephrol Dial Transplant*. 2011;26(11):3581-3588. doi:10.1093/ndt/gfr135.

94. Devarajan P. The use of targeted biomarkers for chronic kidney disease. *Adv Chronic Kidney Dis*. 2010;17(6):469-479. doi:10.1053/j.ackd.2010.09.002.

95. Wei F, Karihaloo A, Yu Z, et al. Neutrophil gelatinase-associated lipocalin suppresses cyst growth by Pkd1 null cells in vitro and in vivo. *Kidney Int*. 2008;74(10):1310-1318. doi:10.1038/ki.2008.395.

96. Schmidt-Ott KM, Mori K, Li JY, et al. Dual action of neutrophil gelatinase-associated lipocalin. *J Am Soc Nephrol*. 2007;18(2):407-413. doi:10.1681/ASN.2006080882.

97. Bolignano D, Donato V, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) as a marker of kidney damage. *Am J Kidney Dis*. 2008;52(3):595-605. doi:10.1053/j.ajkd.2008.01.020.

98. Paragas N, Kulkarni R, Werth M, et al. α-Intercalated cells defend the urinary system from bacterial infection. *J Clin Invest*. 2014;124(7):2963-2976. doi:10.1172/JCI71630.

99. Liu KD, Yang W, Go AS, et al. Urine neutrophil gelatinase-associated lipocalin and risk of cardiovascular disease and death in CKD: results from the Chronic Renal Insufficiency Cohort (CRIC) Study. *Am J Kidney Dis*. 2015;65(2):267-274. doi:10.1053/j.ajkd.2014.07.025.

100. Bolignano D, Coppolino G, Romeo A, Lacquaniti A, Buemi M. Neutrophil gelatinase-associated lipocalin levels in chronic haemodialysis patients. *Nephrology (Carlton)*. 2010;15(1):23-26. doi:10.1111/j.1440-1797.2009.01163.x.

101. Bolignano D, Lacquaniti A, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. *Clin J Am Soc Nephrol*. 2009;4(2):337-344. doi:10.2215/CJN.03530708.

102. Lin HY-H, Hwang D-Y, Lee SC, et al. Urinary neutrophil gelatinase-associated lipocalin and clinical outcomes in chronic kidney disease patients. *Clin Chem Lab Med*. 2015;53(1):73-83. doi:10.1515/cclm-2014-0647.

103. Bolignano D, Coppolino G, Campo S, et al. Neutrophil gelatinase-associated lipocalin in patients with autosomal-dominant polycystic kidney disease. *Am J Nephrol*. 2007;27(4):373-378. doi:10.1159/000103912.

104. Kotsis F, Nitschke R, Boehlke C, Bashkurov M, Walz G, Kuehn EW. Ciliary calcium signaling is modulated by kidney injury molecule-1 (Kim1). *Pflugers Arch*. 2007;453(6):819-829. doi:10.1007/s00424-006-0168-0.

105. Rees AJ, Kain R. Kim-1/Tim-1: from biomarker to therapeutic target? *Nephrol Dial Transplant*. 2008;23(11):3394-3396. doi:10.1093/ndt/gfn480.

106. Kooiman J, van de Peppel WR, Sijpkens YWJ, et al. No increase in Kidney Injury Molecule-1 and Neutrophil Gelatinase-Associated Lipocalin excretion following intravenous contrast enhanced-CT. *Eur Radiol*. March 2015:1-9. doi:10.1007/s00330-015-3624-4.

107. Yang L, Brooks CR, Xiao S, et al. KIM-1-mediated phagocytosis reduces acute injury to the kidney. *J Clin Invest*. 2015;125(125(4)):0-0. doi:10.1172/JCI75417.

108. Humphreys BD, Xu F, Sabbisetti V, et al. Chronic epithelial kidney injury molecule-1 expression causes murine kidney fibrosis. *J Clin Invest*. 2013;123(9):4023-4035. doi:10.1172/JCI45361.

109. Kuehn EW, Hirt MN, John A-K, et al. Kidney injury molecule 1 (Kim1) is a novel ciliary molecule and interactor of polycystin 2. *Biochem Biophys Res Commun*. 2007;364(4):861-866. doi:10.1016/j.bbrc.2007.10.103.

110. Kuehn EW, Park KM, Somlo S, Bonventre JV. Kidney injury molecule-1 expression in murine polycystic kidney disease. *Am J Physiol Renal Physiol*. 2002;283(6):F1326-F1336. doi:10.1152/ajprenal.00166.2002.

111. Nauta FL, Boertien WE, Bakker SJL, et al. Glomerular and tubular damage markers are elevated in patients with diabetes. *Diabetes Care*. 2011;34(4):975-981. doi:10.2337/dc10-1545.

112. Koyner JL, Vaidya VS, Bennett MR, et al. Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. *Clin J Am Soc Nephrol*. 2010;5(12):2154-2165. doi:10.2215/CJN.00740110.

113. Arthur JM, Hill EG, Alge JL, et al. Evaluation of 32 urine biomarkers to predict the progression of acute kidney injury after cardiac surgery. *Kidney Int*. 2014;85(2):431-438. doi:10.1038/ki.2013.333.

114. Bhavsar NA, Köttgen A, Coresh J, Astor BC. Neutrophil gelatinase-associated lipocalin (NGAL) and kidney injury molecule 1 (KIM-1) as predictors of incident CKD stage 3: the Atherosclerosis Risk in Communities (ARIC) Study. *Am J Kidney Dis*. 2012;60(2):233-240. doi:10.1053/j.ajkd.2012.02.336.

115. Rampoldi L, Scolari F, Amoroso A, Ghiggeri G, Devuyst O. The rediscovery of uromodulin (Tamm-Horsfall protein): from tubulointerstitial nephropathy to chronic kidney disease. *Kidney Int*. 2011;80(4):338-347. doi:10.1038/ki.2011.134.

116. Devuyst O, Dahan K, Pirson Y. Tamm-Horsfall protein or uromodulin: new ideas about an old molecule. *Nephrol Dial Transplant*. 2005;20(7):1290-1294. doi:10.1093/ndt/gfh851.

117. Lhotta K. Uromodulin and chronic kidney disease. *Kidney Blood Press Res*.

2010;33(5):393-398. doi:10.1159/000320681.

118. Chakraborty J, Below AA, Solaiman D. Tamm-Horsfall protein in patients with kidney damage and diabetes. *Urol Res*. 2004;32(2):79-83. doi:10.1007/s00240-003-0374-6.

119. Vyletal P, Bleyer AJ, Kmoch S. Uromodulin biology and pathophysiology--an update. *Kidney Blood Press Res*. 2010;33(6):456-475. doi:10.1159/000321013.

120. Kemter E, Rathkolb B, Rozman J, et al. Novel missense mutation of uromodulin in mice causes renal dysfunction with alterations in urea handling, energy, and bone metabolism. *Am J Physiol Renal Physiol*. 2009;297(5):F1391-F1398. doi:10.1152/ajprenal.00261.2009.

121. Scolari F, Caridi G, Rampoldi L, et al. Uromodulin storage diseases: clinical aspects and mechanisms. *Am J Kidney Dis*. 2004;44(6):987-999.

122. Trudu M, Janas S, Lanzani C, et al. Common noncoding UMOD gene variants induce salt-sensitive hypertension and kidney damage by increasing uromodulin expression. *Nat Med*. 2013;19(12):1655-1660. doi:10.1038/nm.3384.

123. Mo L, Zhu X-H, Huang H-Y, Shapiro E, Hasty DL, Wu X-R. Ablation of the Tamm-Horsfall protein gene increases susceptibility of mice to bladder colonization by type 1-fimbriated Escherichia coli. *Am J Physiol Renal Physiol*. 2004;286(4):F795-F802. doi:10.1152/ajprenal.00357.2003.

124. Pak J, Pu Y, Zhang ZT, Hasty DL, Wu XR. Tamm-Horsfall protein binds to type 1 fimbriated Escherichia coli and prevents E. coli from binding to uroplakin Ia and Ib receptors. *J Biol Chem*. 2001;276(13):9924-9930. doi:10.1074/jbc.M008610200.

125. Dahan K, Devuyst O, Smaers M, et al. A cluster of mutations in the UMOD gene causes familial juvenile hyperuricemic nephropathy with abnormal expression of uromodulin. *J Am Soc Nephrol*. 2003;14(11):2883-2893.

126. Prajczer S, Heidenreich U, Pfaller W, Kotanko P, Lhotta K, Jennings P. Evidence for a role of uromodulin in chronic kidney disease progression. *Nephrol Dial Transplant*. 2010;25(6):1896-1903. doi:10.1093/ndt/gfp748.

127. Sejdiu I, Torffvit O. Decreased urinary concentration of Tamm-Horsfall protein is associated with development of renal failure and cardiovascular death within 20 years in type 1 but not in type 2 diabetic patients. *Scand J Urol Nephrol*. 2008;42(2):168-174. doi:10.1080/00365590701644691.

128. Bernard A, Lauwerys R. Low-molecular-weight proteins as markers of organ toxicity with special reference to Clara cell protein. *Toxicology Letters*. 1995;77(1-3):145-151. doi:10.1016/0378-4274(95)03284-3.

129. Lakind JS, Holgate ST, Ownby DR, et al. A critical review of the use of Clara cell secretory protein (CC16) as a biomarker of acute or chronic pulmonary effects. *Biomarkers*. 2007;12(5):445-467. doi:10.1080/13547500701359327.

130. Kabanda A, Jadoul M, Lauwerys R, Bernard A, van Ypersele de Strihou C. Low molecular weight proteinuria in Chinese herbs nephropathy. *Kidney Int*. 1995;48(5):1571-1576.

131. Hermans C, Dong P, Robin M, et al. Determinants of serum levels of surfactant proteins A and B and Clara cell protein CC16. *Biomarkers*. 2003;8(6):461-471. doi:10.1080/1354750031000164702l.

132. Halatek T, Hermans C, Broeckaert F, et al. Quantification of Clara cell protein in rat and mouse biological fluids using a sensitive immunoassay. *Eur Respir J*. 1998;11(3):726-733.

133. Burmeister R, Boe IM, Nykjaer A, et al. A two-receptor pathway for catabolism of Clara cell secretory protein in the kidney. *J Biol Chem*. 2001;276(16):13295-13301. doi:10.1074/jbc.M010679200.

134. Bernard A. Renal dysfunction induced by cadmium: biomarkers of critical effects. *Biometals*. 2004;17(5):519-523.

135. Obermüller N, Kränzlin B, Blum WF, Gretz N, Witzgall R. An endocytosis defect as a possible cause of proteinuria in polycystic kidney disease. *Am J Physiol Renal Physiol*. 2001;280(2):F244-F253.

136. Liebau MC, Serra AL. Looking at the (w)hole: magnet resonance imaging in polycystic kidney disease. *Pediatr Nephrol*. 2013;28(9):1771-1783. doi:10.1007/s00467-012-2370-y.

137. Chapman AB, Bost JE, Torres VE, et al. Kidney volume and functional outcomes in autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol*. 2012;7(3):479-486. doi:10.2215/CJN.09500911.

138. Irazabal MV, Rangel LJ, Bergstralh EJ, et al. Imaging classification of autosomal dominant polycystic kidney disease: a simple model for selecting patients for clinical trials. *J Am Soc Nephrol*. 2015;26(1):160-172. doi:10.1681/ASN.2013101138.

139. Bhutani H, Smith V, Rahbari Oskoui F, et al. A comparison of ultrasound and magnetic resonance imaging shows that kidney length predicts chronic kidney disease in autosomal dominant polycystic kidney disease. *Kidney Int*. April 2015. doi:10.1038/ki.2015.71.

140. Bae KT, Tao C, Wang J, et al. Novel approach to estimate kidney and cyst volumes using mid-slice magnetic resonance images in polycystic kidney disease. *Am J Nephrol*. 2013;38(4):333-341. doi:10.1159/000355375.

141. Grantham JJ, Mulamalla S, Swenson-Fields KI. Why kidneys fail in autosomal dominant polycystic kidney disease. *Nat Rev Nephrol*. 2011;7(10):556-566. doi:10.1038/nrneph.2011.109.

142. Lentine KL, Xiao H, Machnicki G, Gheorghian A, Schnitzler MA. Renal function and

healthcare costs in patients with polycystic kidney disease. *Clin J Am Soc Nephrol*. 2010;5(8):1471-1479. doi:10.2215/CJN.00780110.

143. Torres VE, Harris PC. Autosomal dominant polycystic kidney disease: the last 3 years. *Kidney Int*. 2009;76(2):149-168. doi:10.1038/ki.2009.128.

144. Masyuk TV, Radtke BN, Stroope AJ, et al. Pasireotide is more effective than octreotide in reducing hepatorenal cystogenesis in rodents with polycystic kidney and liver diseases. *Hepatology*. 2013;58(1):409-421. doi:10.1002/hep.26140.

145. Helal I, Reed B, Schrier RW. Emergent early markers of renal progression in autosomal-dominant polycystic kidney disease patients: implications for prevention and treatment. *Am J Nephrol*. 2012;36(2):162-167. doi:10.1159/000341263.

146. Cornec-Le Gall E, Audrézet M-P, Chen J-M, et al. Type of PKD1 mutation influences renal outcome in ADPKD. *J Am Soc Nephrol*. 2013;24(6):1006-1013. doi:10.1681/ASN.2012070650.

147. Chapman AB, Guay-Woodford LM, Grantham JJ, et al. Renal structure in early autosomal-dominant polycystic kidney disease (ADPKD): The Consortium for Radiologic Imaging Studies of Polycystic Kidney Disease (CRISP) cohort. *Kidney Int*. 2003;64(3):1035-1045. doi:10.1046/j.1523-1755.2003.00185.x.

148. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612.

149. Lash JP, Go AS, Appel LJ, et al. Chronic Renal Insufficiency Cohort (CRIC) Study: baseline characteristics and associations with kidney function. *Clin J Am Soc Nephrol*. 2009;4(8):1302-1311. doi:10.2215/CJN.00070109.

150. Imai E, Matsuo S, Makino H, et al. Chronic Kidney Disease Japan Cohort study: baseline characteristics and factors associated with causative diseases and renal function. *Clin Exp Nephrol*. 2010;14(6):558-570. doi:10.1007/s10157-010-0328-6.

151. Eckardt K-U, Bärthlein B, Baid-Agrawal S, et al. The German Chronic Kidney Disease (GCKD) study: design and methods. *Nephrol Dial Transplant*. 2012;27(4):1454-1460. doi:10.1093/ndt/gfr456.

152. Peterson RA, Kimmel PL, Sacks CR, Mesquita ML, Simmens SJ, Reiss D. Depression, perception of illness and mortality in patients with end-stage renal disease. *Int J Psychiatry Med*. 1991;21(4):343-354.

153. Kimmel PL, Weihs K, Peterson RA. Survival in hemodialysis patients: the role of depression. *J Am Soc Nephrol*. 1993;4(1):12-27.

154. Devins GM. Psychosocial issues in end-stage renal disease: introduction. *Adv Ren Replace Ther*. 1994;1(3):195-197.

155. Devuyst O, Antignac C, Bindels RJM, et al. The ERA-EDTA Working Group on

inherited kidney disorders. *Nephrol Dial Transplant*. 2012;27(1):67-69. doi:10.1093/ndt/gfr764.

156. Blanco G, Wallace DP. Novel role of ouabain as a cystogenic factor in autosomal dominant polycystic kidney disease. *Am J Physiol Renal Physiol*. 2013;305(6):F797-F812. doi:10.1152/ajprenal.00248.2013.

157. Briggs JP. The hunt for the perfect biomarker for acute kidney injury: back to gamma-trace? *Kidney Int*. 2008;74(8):987-989. doi:10.1038/ki.2008.426.

158. Singer E, Elger A, Elitok S, et al. Urinary neutrophil gelatinase-associated lipocalin distinguishes pre-renal from intrinsic renal failure and predicts outcomes. *Kidney Int*. 2011;80(4):405-414. doi:10.1038/ki.2011.41.

159. Damman K, Van Veldhuisen DJ, Navis G, et al. Tubular damage in chronic systolic heart failure is associated with reduced survival independent of glomerular filtration rate. *Heart*. 2010;96(16):1297-1302. doi:10.1136/hrt.2010.194878.

160. Aberg F, Lempinen M, Hollmén M, Nordin A, Mäkisalo H, Isoniemi H. Neutrophil gelatinase-associated lipocalin associated with irreversibility of pre-liver transplant kidney dysfunction. *Clin Transplant*. June 2014:n/a–n/a. doi:10.1111/ctr.12394.

161. Nisula S, Yang R, Kaukonen K-M, et al. The Urine Protein NGAL Predicts Renal Replacement Therapy, but Not Acute Kidney Injury or 90-Day Mortality in Critically Ill Adult Patients. *Anesth Analg*. 2014;119(1):95-102. doi:10.1213/ANE.0000000000000243.

162. Haase-Fielitz A, Haase M, Devarajan P. Neutrophil gelatinase-associated lipocalin as a biomarker of acute kidney injury: a critical evaluation of current status. *Ann Clin Biochem*. 2014;51(Pt 3):335-351. doi:10.1177/0004563214521795.

163. Möllsten A, Torffvit O. Tamm-Horsfall protein gene is associated with distal tubular dysfunction in patients with type 1 diabetes. *Scand J Urol Nephrol*. 2010;44(6):438-444. doi:10.3109/00365599.2010.504190.

164. Zhou J, Chen Y, Liu Y, et al. Urinary uromodulin excretion predicts progression of chronic kidney disease resulting from IgA nephropathy. Najafian B, ed. *PLoS ONE*. 2013;8(8):e71023. doi:10.1371/journal.pone.0071023.

165. Bernard AM, Thielemans NO, Lauwerys RR. Urinary protein 1 or Clara cell protein: a new sensitive marker of proximal tubular dysfunction. *Kidney Int Suppl*. 1994;47:S34-S37.

166. Pavik I, Jaeger P, Kistler AD, et al. Patients with autosomal dominant polycystic kidney disease have elevated fibroblast growth factor 23 levels and a renal leak of phosphate. *Kidney Int*. 2011;79(2):234-240. doi:10.1038/ki.2010.375.

167. Youhanna S, Weber J, Beaujean V, Glaudemans B, Sobek J, Devuyst O. Determination of uromodulin in human urine: influence of storage and processing. *Nephrol Dial*

Transplant. 2014;29(1):136-145. doi:10.1093/ndt/gft345.

168. Bernard A, Lauwerys R, Noel A, Vandeleene B, Lambert A. Determination by latex immunoassay of protein 1 in normal and pathological urine. *Clin Chim Acta*. 1991;201(3):231-245.

169. Lohninger H. Lohninger: Grundlagen der Statistik - Google Scholar. *Virtual Institute of Applied Science Von statistic for you …*. 2007.

170. Chao Y-CE, Zhao Y, Kupper LL, Nylander-French LA. Quantifying the relative importance of predictors in multiple linear regression analyses for public health studies. *J Occup Environ Hyg*. 2008;5(8):519-529. doi:10.1080/15459620802225481.

171. Wagenmakers E-J, Farrell S. AIC model selection using Akaike weights. *Psychon Bull Rev*. 2004;11(1):192-196.

172. Lütge C. Zur Ethik von Napster, Gnutella und anderen Internet-Musiktauschbörsen. In: *Wirtschaftsethische Fragen Der E-Economy*. Vol Heidelberg: Physica-Verlag HD; 2003:347-359. doi:10.1007/978-3-642-57345-3_20.

173. Carone FA, Ozono S, Samma S, Kanwar YS, Oyasu R. Renal functional changes in experimental cystic disease are tubular in origin. *Kidney Int*. 1988;33(1):8-13.

174. Gattone VH, Maser RL, Tian C, Rosenberg JM, Branden MG. Developmental expression of urine concentration-associated genes and their altered expression in murine infantile-type polycystic kidney disease. *Dev Genet*. 1999;24(3-4):309-318. doi:10.1002/(SICI)1520-6408(1999)24:3/4<309::AID-DVG14>3.0.CO;2-5.

175. Ho TA, Godefroid N, Gruzon D, et al. Autosomal dominant polycystic kidney disease is associated with central and nephrogenic defects in osmoregulation. *Kidney Int*. 2012;82(10):1121-1129. doi:10.1038/ki.2012.225.

176. Meijer E, Rook M, Tent H, et al. Early renal abnormalities in autosomal dominant polycystic kidney disease. *Clin J Am Soc Nephrol*. 2010;5(6):1091-1098. doi:10.2215/CJN.00360110.

177. Parikh CR, Dahl NK, Chapman AB, et al. Evaluation of urine biomarkers of kidney injury in polycystic kidney disease. *Kidney Int*. 2012;81(8):784-790. doi:10.1038/ki.2011.465.

178. Olden M, Corre T, Hayward C, et al. Common variants in UMOD associate with urinary uromodulin levels: a meta-analysis. *J Am Soc Nephrol*. 2014;25(8):1869-1882. doi:10.1681/ASN.2013070781.

179. Devuyst O, Thakker RV. Dent's disease. *Orphanet J Rare Dis*. 2010;5(1):28. doi:10.1186/1750-1172-5-28.

180. Jouret F, Bernard A, Hermans C, et al. Cystic fibrosis is associated with a defect in apical receptor-mediated endocytosis in mouse and human kidney. *J Am Soc Nephrol*.

2007;18(3):707-718. doi:10.1681/ASN.2006030269.

181. Tracz MJ, Alam J, Nath KA. Physiology and pathophysiology of heme: implications for kidney disease. *J Am Soc Nephrol*. 2007;18(2):414-420. doi:10.1681/ASN.2006080894.

182. Dedi R, Bhandari S, Turney JH, Brownjohn AM, Eardley I. Lesson of the week: Causes of haematuria in adult polycystic kidney disease. *BMJ*. 2001;323(7309):386-387.

183. European Confederation of Laboratory Medicine. European urinalysis guidelines. *Scand J Clin Lab Invest Suppl*. 2000;231:1-86.

184. Grossfeld GD, Litwin MS, Wolf JS, et al. Evaluation of asymptomatic microscopic hematuria in adults: the American Urological Association best practice policy--part II: patient evaluation, cytology, voided markers, imaging, cystoscopy, nephrology evaluation, and follow-up. *Urology*. 2001;57(4):604-610.

185. Myint TM, Rangan GK, Webster AC. Treatments to slow progression of autosomal dominant polycystic kidney disease: systematic review and meta-analysis of randomized trials. *Nephrology (Carlton)*. 2014;19(4):217-226. doi:10.1111/nep.12211.

186. Spithoven EM, Kramer A, Meijer E, et al. Analysis of data from the ERA-EDTA Registry indicates that conventional treatments for chronic kidney disease do not reduce the need for renal replacement therapy in autosomal dominant polycystic kidney disease. *Kidney Int*. 2014;86(6):1244-1252. doi:10.1038/ki.2014.120.

187. Reule S, Sexton DJ, Solid CA, Chen S-C, Collins AJ, Foley RN. ESRD from autosomal dominant polycystic kidney disease in the United States, 2001-2010. *Am J Kidney Dis*. 2014;64(4):592-599. doi:10.1053/j.ajkd.2014.05.020.

188. Orskov B, Rømming Sørensen V, Feldt-Rasmussen B, Strandgaard S. Improved prognosis in patients with autosomal dominant polycystic kidney disease in Denmark. *Clin J Am Soc Nephrol*. 2010;5(11):2034-2039. doi:10.2215/CJN.01460210.

189. Rizk D, Jurkovitz C, Veledar E, et al. Quality of life in autosomal dominant polycystic kidney disease patients not yet on dialysis. *Clin J Am Soc Nephrol*. 2009;4(3):560-566. doi:10.2215/CJN.02410508.

190. Mapes DL, Bragg-Gresham JL, Bommer J, et al. Health-related quality of life in the Dialysis Outcomes and Practice Patterns Study (DOPPS). *Am J Kidney Dis*. 2004;44(5 Suppl 2):54-60.

191. Chapman AB. Approaches to testing new treatments in autosomal dominant polycystic kidney disease: insights from the CRISP and HALT-PKD studies. *Clin J Am Soc Nephrol*. 2008;3(4):1197-1204. doi:10.2215/CJN.00060108.

192. Chapin HC, Caplan MJ. The cell biology of polycystic kidney disease. *J Cell Biol*. 2010;191(4):701-710. doi:10.1083/jcb.201006173.

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